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THE ANTIGONADOTROPIC HORMONES IN NORMAL HUMAN BLOOD SERUM[1]

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THE PHYSIOLOGICAL responses to certain hormones are complicated by the development of resistant substances, the so-called antihormones. At present the most clearly defined of these is the antithyroid substance described by Blum (1). Although Collip and Selye et al. (2) have succeeded in developing inhibitory substances in the blood of animals injected with large amounts of gonadotropic extracts over long periods of time, such antihormones have never been shown to occur normally in human blood serum.

The present problem is concerned with the activating effect of the gonadotropic substances on the growth of the immature rat ovary. The specific question investigated was whether or not an antigonadotropic hormone normally exists in human blood serum.

EXPERIMENTAL PROCEDURE

Human pituitary glands were obtained at autopsy and dehydrated overnight in 98% acetone. The anterior lobes were separated out and ground to a fine powder. Fifty glands were pooled, uniformly mixed and stored in the ice box at 4° C. to be used as the stock material for the preparation of the standard gonadotropic extract. A fresh extract was made before each series of injections by shaking 3 mg. per cc. of dry powder with distilled water, allowing any insoluble material to settle overnight. The supernatant liquid was then poured off and used as the standard extract.

Three hundred ninety-three rats of Wistar stock, 19 to 24 days old and weighing 35 to 45 gm., served as experimental animals. After weaning, they were fed a stock diet of Purina fox chow and lettuce, adequate in all respects for normal maintenance and growth. The animals were kept in large cages (not more than 8 to a cage) and had free access to food and water.

Blood for the experiments was obtained by venipuncture from 5 normal human females. It was allowed to stand for 30 minutes, after which time a clot had formed. The blood was then centrifuged at medium speed for 10 minutes, the serum pipetted off and stored in the ice box at 4° C. to be used for injections over a period of 5 days.

[1] This work was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry in the School of Medicine and Dentistry of the University of Rochester, Rochester, N. Y.

The experimental animals were divided into 4 groups and kept under comparable conditions except for the treatments outlined in the following schedule.

Group 1 Untreated controls received no injections.
Group 2 Serum-injected controls, received a total of 5 cc. of human blood serum in doses of 0.5 cc. twice daily for 5 days.
Group 3 Extract injected controls which were subdivided into
Subgroup 3*a* received a total of 0.6 cc. of extract in doses of 0.1 cc. twice daily for 3 days.
Subgroup 3*b* received a total of 0.8 cc. of extract in doses of 0.1 cc. twice daily for 4 days.
Group 4 Experimental animals received a combination of serum and extract, and subdivided into
Subgroup 4*a* received a total of 5 cc. of human blood serum in 0.5 cc. doses twice daily for 5 days, plus a total of 0.6 cc. of extract in doses of 0.1 cc. twice daily for 3 days.
Subgroup 4*b* received a total of 5 cc. of human blood serum in doses of 0.5 cc. twice daily for 5 days, plus a total of 0.8 cc. of extract in doses of 0.1 cc. twice daily for 4 days.

Histological sections were made of the ovaries of a series of animals from each of the 4 groups described. These sections were fixed in Zenker's solution, mounted in

TABLE 1. PRELIMINARY EXTRACT-INJECTED CONTROLS

Group	No. of rats	Age	Dosage	Ave. paired ovarian wt.
		days		mg.
c	14	19	0.1 cc. twice daily for 4 days	33.7±15.7[1]
	19	23		76.9±22.0
	11	24		106.9±36.4
d	11	23	0.2 cc. once daily for 4 days	16.0± 4.7
	7	26		39.6±11.0
e	5	19	0.4 cc. once daily for 2 days	18.0± 7.6
	11	24		36.1±11.4
f	8	19	0.8 cc. in one injection	17.5± 6.3
	5	24		27.2±13.8
Total	91	19–26		

All rats received a total of 0.8 cc. aqueous extract (equivalent to 2.4 mg. dry powder) of human anterior pituitary lobe.
[1] Average values—average deviation.

paraffin and stained by hematoxylin and eosin. They were studied for evidences of maturation of the graafian follicle and luteinization.

Considerable work was done to establish an optimum frequency of injection; the optimum dosages of extract; the age of rats best suited for these experiments and the potency of the extract. Four preliminary groups of animals were injected as follows: (*c*), 0.1 cc. twice daily for 4 days; (*d*), 0.2 cc. once daily for 4 days; (*e*), 0.4 cc. once daily for 2 days; (*f*), 0.8 cc. in one injection. In each of these groups, animals of different ages were included. The animals in groups (*d*), (*e*) and (*f*) and also the 19-day old animals in group (*c*) gave paired ovarian weights of 16.0 to 39.6 mg. (table 1). In contrast the 23-day-old animals showed an average paired ovarian weight of 76.9 ±22.0 mg. while the 24-day-old group gave an average paired ovarian weight of 106.9±36.4 mg. It is apparent that the older animals (23 to 24 days) showed a much greater average paired ovarian weight than younger animals injected in the same manner, or animals of the same age given the same amount of extract in fewer injections. The greater weight change was a better index of stimulation, therefore all of the experimental groups 3*a*, 3*b*, 4*a* and 4*b* were taken at 23 to 24 days, and injected twice daily with 0.1 cc. of extract at approximately the same hour each day for 3 or 4 days.

TABLE 2. SUMMARY DATA OF EXPERIMENTAL GROUPS

Group	No. of rats	Dosage		Ave. paired ovarian wt.
		Serum	Extract	
				mg.
1	56	None	None	13.6± 2.6
2	54	5 cc.	None	13.3± 3.2
3a	45	None	0.6 cc.	46.7±15.9
3b	27	None	0.8 cc.	98.6±29.1
4a	68	5 cc.	0.6 cc.	40.7±16.6
4b	99	5 cc.	0.8 cc.	79.9±27.5
Total	349			

All rats were 23 to 24 days old.
The human anterior pituitary lobe extract contained 3 mg. of dry powder per cc.

For comparable results in all animals, they were considered to be experimental for 8 days. The animals of Group 2 were injected with serum on the 1st, 2nd, 3rd, 4th and 5th days, rested for 2 days and were killed on the morning of the 8th day. Groups 3*a* and 3*b* were likewise injected with extract on the 3rd, 4th, and 5th days and killed on the 8th day. Groups 4*a* and 4*b* were treated by a combination of these two procedures and killed on the 8th day (table 2).

In order to compare the potency of the human anterior pituitary extract given the rats with the potency as obtained in the Aschheim-Zondek tests, the smallest amount of extract producing ovulation in the mature female rabbit was determined. A dose of 0.2 cc. of aqueous extract or an equivalent of 0.6 mg. of the fresh dried anterior lobe powder caused the graafian follicles to rupture in 16 to 18 hours. Considering one rabbit unit as the amount causing ovulation, the potency of the powder used was calculated as approximately 2 RB.U. per mg. Thus, the 2 usual amounts given (table 2) may be expressed in terms of rabbit units as follows: 0.6 cc. equivalent to 3.6 RB.U. or 1.8 mg. dry anterior lobe powder; 0.8 cc. equivalent to 4.8 RB.U. or 2.4 mg. dry anterior lobe powder.

In addition to the rats in tables 1 and 2, a few were given much larger doses in fewer injections. The results tabulated in table 3 indicate that the previous choices of frequency of injection, and dosage level were more satisfactory than large amounts in fewer injections.

RESULTS

The results may be followed by referring to the tabulated procedure and the summary data (table 2), where the number of rats, ages and paired ovarian weights are given. The animals in Group 2 which received serum from normal human females showed no difference in ovary weights as compared to the 'no-treatment' control

TABLE 3

No. of rats	Daily dose in cc.	No. of doses	Equivalent in mg. of dry anterior lobe powder	Ave. paired ovarian weights in mg.
2	0.6	3	5.4	100.0
4	0.8	3	7.2	65.9
Average from table 2				
27	0.2 (in 2 doses)	8	2.4	98.6

animals of Group 1. The animals in Group 3 which received human anterior pituitary extract showed the expected response of a marked increase in ovary weights. If the serum from normal human females contains an antigonadotropic substance, the simultaneous injection of serum and anterior pituitary extract (Group 4) should result in an inhibition of the growth response to the extract.

Group 1. These animals were controls and received no treatment. It was found that a total of 100 rats ranging in ages from 19 to 24 days (27 to 32 days when killed) gave an average paired ovarian weight of 12.2 ± 2.9 mg. In general rats of these ages varied little in their paired ovarian weights although their body weights might range from 25 to 80 gm. The lowest average paired ovarian weight was 7.0 mg. observed

TABLE 4. EXPERIMENTAL ANIMALS INJECTED WITH A COMBINATION OF SERUM AND EXTRACT

Serum source	Complete menstrual cycle		0–9th day of cycle		10th–14th day of cycle		15th–31st day of cycle	
	No. of rats	Ave. paired ovarian wt.	No. of rats	Ave. paired ovarian wt.	No. of rats	Ave. paired ovarian wt.	No. of rats	Ave. paired ovarian wt.
		mg.		mg.		mg.		mg.
M.D.	95	64.7± 29.1	28	76.1±26.6	17	102.5±27.6	50	45.4±19.6
H. V.	23	47.9±31.3	15	25.1± 8.4	—	— —	8	90.8± 9.8
L. W.	13	66.5±15.5	9	69.3±13.6	4	60.1±15.0	—	— —
A. M.	13	58.2±25.0	4	44.0±10.0	5	46.0± 9.4	4	99.0±11.0
M. O.	23	47.4±19.5	15	39.5±16.3	—	— —	8	62.3±15.0
Total	167	59.6±27.5	71	54.9±14.9	26	83.4±14.0	70	55.6±13.8

in a group of four 20-day-old rats, while the highest was 14.3 ± 2.0 mg. in a group of thirty-three 23-day-old rats.

Group 2. These animals received 5 cc. of human blood serum in 10 equally divided doses of 0.5 cc. twice daily for 5 days. The serum was taken from 5 normal human females who also furnished the serum for the experiments on Group 4. The amount of serum, the number of rats and average paired ovarian weights are given in table 2. A total of 54 animals showed an average paired ovarian weight of 13.3 ± 3.2 mg. The lowest average weight obtained was 11.2 mg. and the highest 14.7 mg. The average paired ovarian weight of the serum controls is almost identical with the similar average from the untreated controls of Group 1.

Group 3. The 45 animals in Group 3*a* received 0.6 cc. of extract (equivalent to 1.8 mg. of dry anterior lobe powder) in doses of 0.1 cc. twice daily for 3 days. They gave an average paired ovarian weight of 46.7 ± 15.9 mg. (table 2). The 27 animals of Group 3*b* were given 0.8 cc. of extract (equivalent to 2.4 mg. of the dry anterior lobe powder) in doses of 0.1 cc. twice daily for 4 days. These animals showed an average paired ovarian weight of 98.6 ± 29.1 mg. which was a great deal larger than the preceding group. The average paired ovarian weight for Group 3*b* showed an 8-fold increase over the similar average for the untreated controls of Group 1.

Group 4. The 68 experimental animals of Group 4*a* were given a combination of 0.6 cc. of extract in 0.1 cc. doses twice daily for 3 days, plus 5 cc. of serum in 0.5 cc. doses twice daily for 5 days. The average paired ovarian weight was 40.7 ± 16.6 mg. (table 2). Group 3*a* which received the same amount of extract only, showed an average paired ovarian weight of 46.7 ± 15.9 mg. The smaller average weight of the Group 4*a* animals (47 − 41 = 6) suggests a slight inhibitory action of the serum. However, the large individual variation, as shown by the large deviations for each average, makes the difference of the two averages not statistically significant.

The animals of Group *4b* received a combination of 0.8 cc. of extract in 0.1 cc. doses twice daily for 4 days, plus 5 cc. of serum in 0.5 cc. doses twice daily for 5 days. They showed an average paired ovarian weight of 79.9 ± 23.7 mg. (table 2). Group *3b* which received a total of 0.8 cc. of extract only, showed an average paired ovarian weight of 98.6 ± 29.1 mg. The smaller average weight of the Group *4b* animals ($97 - 80 = 17$) again suggests an inhibitory action of the serum, but again the difference is not statistically significant, (3).

A further classification of the data of Group *4* is possible since the sera were obtained from 5 individuals during known periods of the menstrual cycle. In table 4, column 1, the data from Group *4a* and *4b* are averaged for each individual. The average paired ovarian weights vary from 47.4 to 66.5 mg. It is impossible to say whether this variation is to be attributed to patient differences or to animal variations.

In the last 3 columns of table 4, the data for each individual are divided into 3 groups related to the period of the menstrual cycle as follows: (*a*) date of menstruation to the 9th day, (*b*) the 10th to the 14th day inclusive, and (*c*) the 15th day to the next date of menstruation. This division was based on the work of Siebke (4, 5) discussed later. In only one case (*M.D.*) were there enough animals in each division to justify a comparison. Here, the average paired ovarian weights were as follows: 0 to 9 days 76, 10 to 14 days 103, 15 to 31 days 45, showing an increase in the mid-menstrual period with a decrease in the final period. Due to the large animal variations, even these differences are not clearly significant statistically. There was no regularity of difference between the average paired ovarian weights in the 3 disivions for the 5 individuals.

The histological sections showed normal ovarian structure for the animals of Group *1a* and *2*. The animals receiving gonadotropic extracts (Group 3 and 4) showed both maturation of the graafian follicle and leutinization, evidence of the changes accompanying the increase in ovary weight. These changes were typical of those repeatedly illustrated in the papers of previous investigators and therefore photomicrographs were not included.

A total of 700 animals was studied and each of these was carefully examined for any necrotic areas at the site of injection which might influence the effects of the injected materials. Such necrosis occurred only rarely and was always limited to a very small area, therefore it cannot be said to have distorted the results.

DISCUSSION

The immature female white rat seemed the most reliable test animal for use in a study of the gonadotropic substances. In the case of the female, sexual maturity is known to supervene before the end of the second month of life. It was found in this study that 23 to 24-day-old rats responded most readily to the injection of aqueous anterior lobe extracts, in contrast to Evans (6) who chose animals 19 to 21 days old. The stimulation of ovary growth with this extract was shown by histological section to be due to both maturation of follicles and/or, in many cases, the formation of corpora lutea. In some cases there was rapid growth of numerous follicles, some of which luteinized while others underwent atresia, so that 96 to 168 hours after the first injection the ovary showed numerous small corpora lutea as well as many follicles, probably in the process of maturation. In a majority of cases there was an enlargement of the uterus accompanying the ovarian change.

The first external evidence of ovarian stimulation was shown by the opening of the vaginal orifice which usually occurred on the 3rd day of injection. Since these changes were secondary to ovarian changes they could be considered significant only

if it had been very clearly demonstrated that the material tested contained no estrogenic substance. This seemed probable since water extracts were used and the estrogenic substances are not water-soluble.

The injection of human blood serum into immature female white rats caused no histological change in the type of cell found in their ovaries as differentiated from those in the normal controls which were given no injections. The paired ovarian weights of these serum-treated rats were practically unchanged from those of the controls, showing no perceptible activation or inhibition either macroscopically or microscopically. By the injection of a combination of the anterior pituitary extract

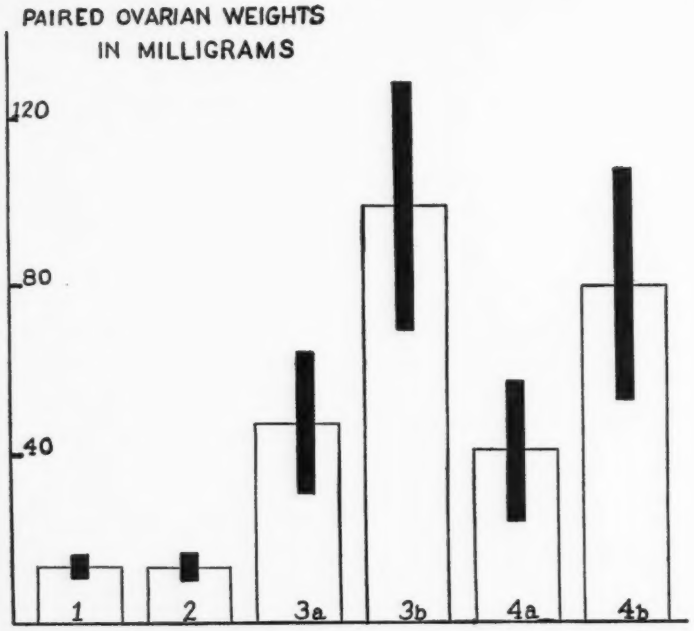

Fig. 1. Height of columns represents average paired ovarian weights of: Group 1. Controls; Group 2. Serum-injected controls; Group 3*a*. Extract-injected (0.6 cc.); Group 3*b*. Extract-injected (0.8 cc.); Group 4*a*. Extract-injected (0.6 cc.) plus 5 cc. serum; Group 4*b*. Extract-injected (0.8 cc.) plus 5 cc. serum. Black columns represent the average deviation of respective groups.

and normal human blood serum an inhibition of the ovarian growth attributable to the injected serum would seem to indicate the presence of an antihormone substance in normal human blood serum. However, since the slight inhibition found was not statistically significant in view of the large animal variations, this theory did not seem plausible. The ovaries of the group given the combination treatment appeared little changed histologically and weighed slightly less than those of the animals receiving only the anterior pituitary extract. These findings might be interpreted as indicating the presence of a hormone-antihormone balance in normal human blood serum, in which case no physiological response could be demonstrated because of the injection of a 'balanced' serum.

Figure 1 presents the results on the 4 groups of animals. It may be seen that the

extract produced an increase in average paired ovarian weights (as compared to the untreated animals of Group 1) of 28 mg. on the 0.6 cc. dosage and 87 mg. on the 0.8 cc. dosage. These increases, attributable to the human anterior pituitary extracts, are closely similar to the increases previously reported using anterior pituitary extracts from various animals, such as the ox, sheep and sow. Comparing the average paired ovarian weights of the animals receiving serum to those receiving the combination of serum plus the two levels of extract showed an increase in the average weights of 34 mg. on the 0.6 cc. dosage and 67 mg. on the 0.8 cc. dosage. The increase due to (0.8 cc.) extract was 87 mg.; the increase due to the same amount of extract in the group of animals also receiving serum was 67 mg. This difference, 20 mg., suggests an inhibitory action of the serum. However, even though the difference amounts to 23% of the increase without serum, it is not statistically significant due to the wide individual variation in the animals.

It has been shown by Siebke (4, 5) that the estrin in the blood of normally menstruating women is low at the menstrual period, gradually increasing in amount up to the 14th day of the menstrual cycle. He suggested that prolan A stimulates the ovarian follicle with resultant estrin production, supposedly at the time of ovulation, perhaps due to the influence of the pituitary gland. The level of estrin again rises only to fall rapidly just before the onset of the menstrual period.

If antihormones are a physiological entity and play a part in the mechanism of normal menstruation in the human being, the amount of antihormone in the blood should vary during different periods of the menstrual cycle, perhaps paralleling the hormone content. The data presented in table 4 show no uniformity of variation, either inhibitory or stimulatory, when blood sera of the early, middle or late periods of the menstrual cycle are injected along with gonadotropic extracts. If the greatest concentration of gonadal substances is normally present in the mid-period, then the presence of antihormones might be expected in greatest concentration simultaneously. Viewed in this light, the failure to obtain increases in the ovary weights of the rats receiving sera from *L.W.* and *A.M.* suggests an inhibitory action and contradictorily, the marked rise in weight with the sera of *M-D.* suggests only stimulation. The individuals from whom the sera were taken were chosen as being normal, having regular 28 to 31-day cycles with normal menstrual flow. However, no clear-cut evidence of the occurrence of an antihormone in normal human blood serum can be drawn from the data.

As has been noted, Selye et al. (7) used very large daily injections of anterior pituitary extract (10 to 40 R.U.) over long experimental periods (25 to 60 days) and produced in rats a serum which showed an inhibitory action when this serum and similar doses of extract were injected into a second animal. They concluded that the chronic administration of gonadotropic extracts from the anterior lobe of the pituitary or pregnancy urine led to the formation of substances inhibitory to their action and that passive resistance to extracts from both sources could be established by administration of sera from animals chronically injected with the original extracts. The absence of clear-cut evidence of inhibition by normal human blood serum reported here makes the normal presence of detectable amounts of an antihormone questionable.

SUMMARY

A total of 100 control rats (no treatment, aged 19 to 24 days, gave paired ovarian weights (mg.) as follows: average 12.9 ± 2.9; maximum 14.3 ± 2.0; minimum 7.0 ± 0.0. A group of 54 serum control rats (5 cc. serum), aged 23 to 24 days, gave paired ovarian weights (mg.) as follows: average 13.3 ± 3.2; maximum 14.7 ± 4.6; minimum 11.2 ± 2.5. Thus normal human blood serum taken during various stages of the menstrual

cycle and injected into immature female white rats failed to produce any inhibition or activation of the growth of the ovaries.

A distilled water extract of human anterior lobe powder exerted a marked gonadotropic effect when given in small doses over a period of several days. The best response was obtained with 0.8 cc. of the aqueous extract when given in 8 equal doses, injected twice daily for 4 days. Immature female white rats 23 to 24 days old were found to be the most susceptible to this treatment.

The 72 rats, aged 23 to 24 days, receiving human anterior pituitary extract gave paired ovarian weights (mg.), as follows: (*a*) dose 0.6 cc.; average 46.7 ± 15.9; (*b*) dose 0.8 cc.; average 96.8 ± 29.1. The 167 rats, aged 23 to 24 days, receiving serum (5 cc.) and human anterior pituitary extract, gave paired ovarian weights (mg.), as follows: (*a*) extract dose 0.6 cc.; average 40.7 ± 16.6; (*b*) extract dose 0.8 cc.; average 79.7 ± 23.7. There is no clear-cut evidence that human blood serum normally contains a substance (detectible by our present methods of assay) which exerts an inhibitory effect on human anterior pituitary extract, when both are injected into the experimental animal. The possibility of excess antihormones in the blood of pathological endocrine cases is being studied, and will be reported later.

The writer acknowledges her debt of gratitude to Dr. C. Arthur Elden for his encouragement and guidance so generously given during the many months of these experiments.

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THE EFFECTS OF TESTOSTERONE PROPIONATE ON EPIPHYSEAL CLOSURE, SODIUM AND CHLORIDE BALANCE AND ON SPERM COUNTS

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UNTIL MAY, 1938, we (1) had not seen in a case of eunuchoidism, epiphyseal development which advanced beyond the normal rate during treatment with testis hormone. However, it now appears that large doses of testosterone propionate can cause epiphyseal union to take place at a more rapid rate than normal. Table 1, *case 1* shows that doses of testosterone propionate varying from 15 mg. to 60 mg. per week for 15 months caused no distinct advancement in epiphyseal age but subsequently doses of 105 mg. or more per week caused an increase of about two years in epiphyseal age over a period of 8 months.

TABLE 1. EPIPHYSEAL DEVELOPMENT IN HYPOGONADISM. *Case 1*

Year	Month	Medication	Chronological age	Bone age
1934	October		23	16
1935	July	Prephysin[1]		
1936	January	Prephysin plus antuitrin-S	25	16
	March	Androsterone		
	May	Testosterone		
	August		25	16
	December	Testosterone propionate,[2] 15 mg. per week		
1937	February	30 mg. per week		
	August	60 mg. per week		
	October	60 mg. per week	26	16+
1938	May	105 mg. per week	27	16+
	June	150 mg. per week		
1939	January	150 mg. per week	28	18+

[1] Horse pituitary extract (Chappel & Co.).
[2] Oreton (Schering Corporation).

Table 2, *case 2*, illustrates the extremely slow epiphyseal union advancing from 20 to 21 years over a 6-year period in a case of severe eunuchoidism with inadequate or no therapy. This case is included as one in which the patient had comparable epiphyseal development but was not given active therapy. It may be compared to *cases 3* and *4*.

In *case 3* epiphyseal age advanced very little during 10 months, although testosterone propionate was given during approximately 6 months of this time. Subsequently, during 7 months of therapy, using 75 mg. of testosterone propionate weekly, there was an advance of about one year in epiphyseal development.

In *case 4* an even greater advance was seen, the bone age being calculated as 17 years in April, 1938. The continuance of 60 mg. weekly of testosterone propionate caused an advance of 3 years in bone age within the next 9 months.

TABLE 2. EPIPHYSEAL DEVELOPMENT IN HYPOGONADISM

Year	Month	Medication	Chronological age	Bone age
		Case 2, H.D.		
1932	December	Androtin[1]	32	20
1933	October	Androtin	33	20
1935	February	No treatment	34	20+
1938	January	No treatment	37	21
		Case 3, S.T.		
1936	December		30	20
1937	February	Antuitrin-S,[2] 600 U weekly		
	April	Testosterone propionate,[3] 30 mg. per week		
	August	75 mg. per week		
	October	75 mg. per week	31	20
1938	May	75 mg. per week	31	21
		Case 4, T.M.		
1937	November	Testosterone propionate, 30 mg. per week	33	
1938	January	60 mg. per week		
	April	60 mg. per week	34	17
	September	75 mg. per week		
1939	January	75 mg. per week	34	20+

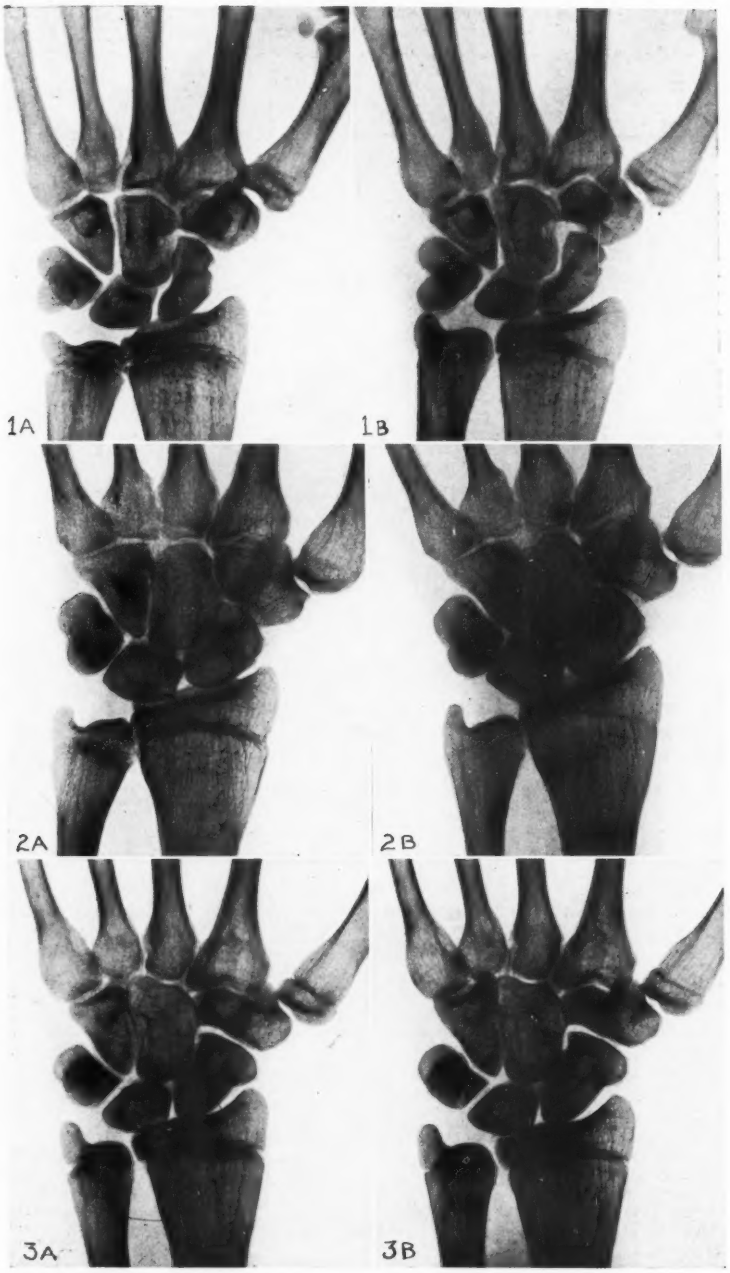
[1] Androtin was a urinary extract of relatively low androgenic potency made in our laboratories.
[2] Pregnancy urine extract (Parke, Davis & Co.)
[3] Oreton (Schering Corporation).

Sodium Chloride and Water Metabolism and Body Weight

Kenyon et al. (2) have reported a weight gain and sodium and nitrogen retention in eunuchoids treated with testosterone propionate. We have studied 2 patients from this viewpoint. The first was 65 years of age and had symptoms which we suspected to have been due to functional testicular deficiency. The 24-hour urinary androgen excretion was 22 international units. The genitalia and secondary sexual characteristics were normal. He was given a 2388-calorie diet, containing 130 C, 80 P and 172 F. The daily nitrogen, sodium and chloride intakes were fixed at 12.8, 1.111 and 1.985 gm. respectively by giving an exact duplication of the same foods in the same quantity daily. This was maintained over a 3-day control period and 6 days of treatment with 50 mg. of testosterone propionate daily. The findings are shown in table 3 (*case 5*).

It is obvious from the chart that there is a definite increase in weight paralleling treatment. Likewise, there is a definite decrease in the urinary output of sodium and to a less extent of chloride, indicating retention of these elements. However, there was no consistent change shown in the urinary nitrogen.

Fig. 1. *Case 1*, A. May, 1938. Epiphyseal development normal for 16 years (chronological age 27) as shown here had changed very little during the preceding 4 years with treatment as indicated in table 1. B. Eight months later after doses of testosterone propionate of 105 to 150 mg. per week epiphyseal maturity approximates the normal for 18 years of age, an advance in epiphyseal development of 2 years in 8 months. Fig. 2. *Case 2*, A. December, 1932. Incomplete epiphyseal union at the lower end of the radius is seen. B. January, 1938. Complete epiphyseal union has occurred. This took place gradually during inadequate or no therapy in the intervening 6 years. Fig. 3. *Case 4*, A. April, 1938. Chronological age of patient 34 years. Epiphyseal age approximately 17 years. B. January, 1939. Nine months later following doses of testosterone propionate of 60 to 76 mg. per week there has been a distinct advance in epiphyseal maturity to a stage approximating normal for 20 years of age, an advance of epiphyseal age of about 3 years in 9 months.



Figs. 1 to 3. (See explanation on preceding page)

TABLE 3. *Case 5.* THE EFFECT OF TESTOSTERONE PROPIONATE ON SODIUM CHLORIDE AND NITROGEN EXCRETION AND ON BODY WEIGHT

Date	Weight	24 hr. intake fluids	24 hr. output of urine					
			Fluids	N	Na	Na	Cl	Cl
		cc.	cc.	gm.	mg. %	gm.	mg. %	gm.
1-27-39	146	2000	790	4.98	235	1.856	650	5.135
1-28-39	146¼	3000	2300	14.81	189	4.347	340	7.820
1-29-39	145¼	2600	1080	9.07	248	2.678	720	7.776
25 mg. of testosterone propionate, intramuscularly, twice daily started on 1/30/39								
1-30-39	146¾	2950	930	8.46	170.2	1.512	650	6.045
1-31-39	148¼	2950	1810	8.87	138	2.664	525	9.507
2- 1-39	148½	1750	1170	8.52	138	1.614	530	6.202
2- 2-39	149½	2930	1600	7.39	55.4	0.886	330	5.280
2- 3-39	150	2600	1030	7.79	64.5	0.664	360	3.708
2- 4-39	150	2400	1120	8.94	58.2	0.651	365	4.088

The second case is that of a 64-year-old male castrate. He was placed on the same fixed diet as that used in the preceding case. During the first 3 days he received no treatment. Subsequently he was given 50 mg. of testosterone propionate intramuscularly daily (table 4, *case 6*). It is interesting to note that the average daily total sodium excretion prior to treatment was 0.89 gm., during treatment 0.39 gm. The total daily chloride excretion averaged 3.49 gm. prior to therapy, 1.82 gm. during therapy. There was a distinct gain in body weight during the test. In this respect it is interesting to note that weight gain during treatment with testosterone propionate is the rule. No consistent change in total N excretion was detected.

Effect on Sperm Counts

Figures 4 to 9 are self-explanatory. In each of the 3 cases shown there was a decided drop in the total number of sperm produced during the period of treatment with testosterone propionate. The decrease in number of sperm cells was so marked in *case 7* (fig. 4) that on the last 4 occasions no sperm cells could be found. In some cases such as 8 and 9, figures 6 and 8 the original drop was followed by a rise while treatment was in progress but in no case did the rise reach the original high level found prior to treatment.

TABLE 4. *Case 6.* THE EFFECT OF TESTOSTERONE PROPIONATE ON SODIUM CHLORIDE AND NITROGEN EXCRETION AND ON BODY WEIGHT

Date	Weight	24 hr. intake fluids	24 hour output of urine					
			Fluids	N	Na	Na	Cl	Cl
		cc.	cc.	gm.	mg. %	gm.	mg. %	gm.
3-11-39		2650	1500	9.87	103	1.545	390	5.850
3-12-39		2400	650	8.60	75	0.487	300	1.950
3-13-39		2200	790	3.70	81	0.639	340	2.686
Intramuscular testosterone propionate, 50 mg. per day begun 3/13/39								
3-15-39	134	2100	790	6.8	35	0.276	225	1.777
3-16-39		1800	515	3.9	107	0.551	345	1.776
3-17-39		2200	780	7.2	61	0.475	315	2.457
3-18-39	134⅓	2100	590	5.3	41	0.241	150	0.885
3-19-39		2000	1320	6.6	28	0.369	115	1.518
3-21-39	137	1825	875	5.0	47	0.411	250	2.187
3-22-39	139	1825	875	5.0	47	0.411	250	2.187

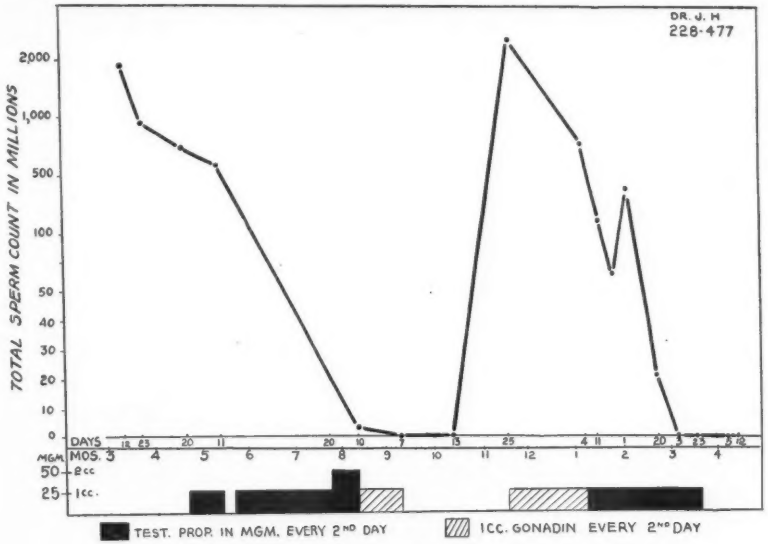


Fig. 4. The effect of testosterone propionate on the sperm count in a case of necrospermia

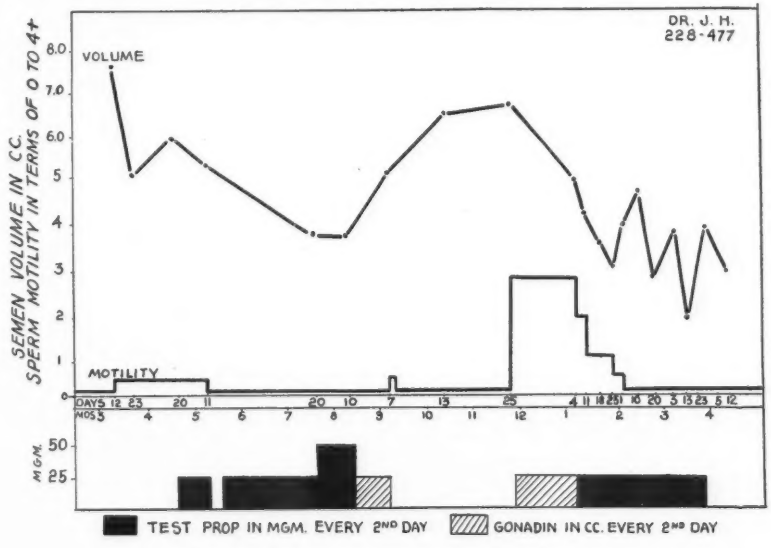


Fig. 5. The effect of testosterone propionate on semen volume and sperm motility in a case of necrospermia.

In *case 7* (fig. 4, 5) sperm motility was very slight but was present preceding the treatment. It is interesting to note that there was a rather marked increase in this after cessation of therapy for over 2 months. The 3 plus motility as shown on figure 5 was present before treatment with pregnant mare's serum (gonadin) was begun

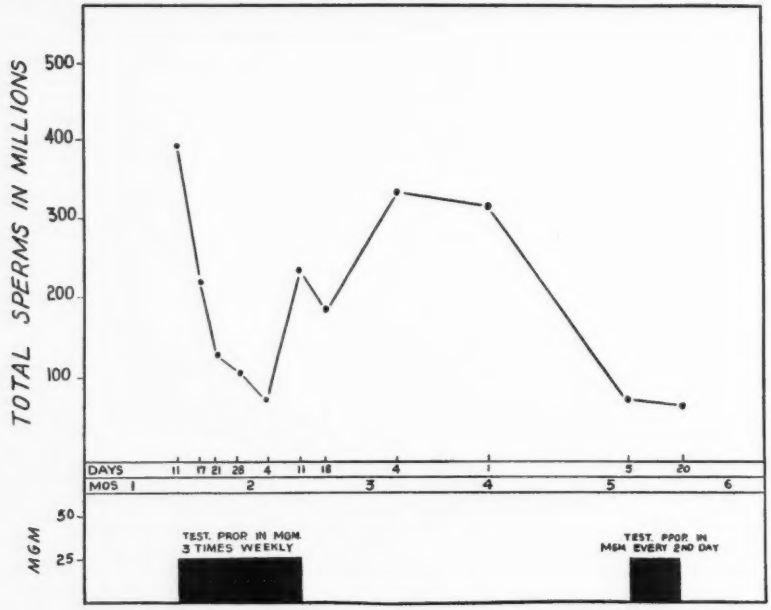


Fig. 6. The effect of testosterone propionate on the sperm count.

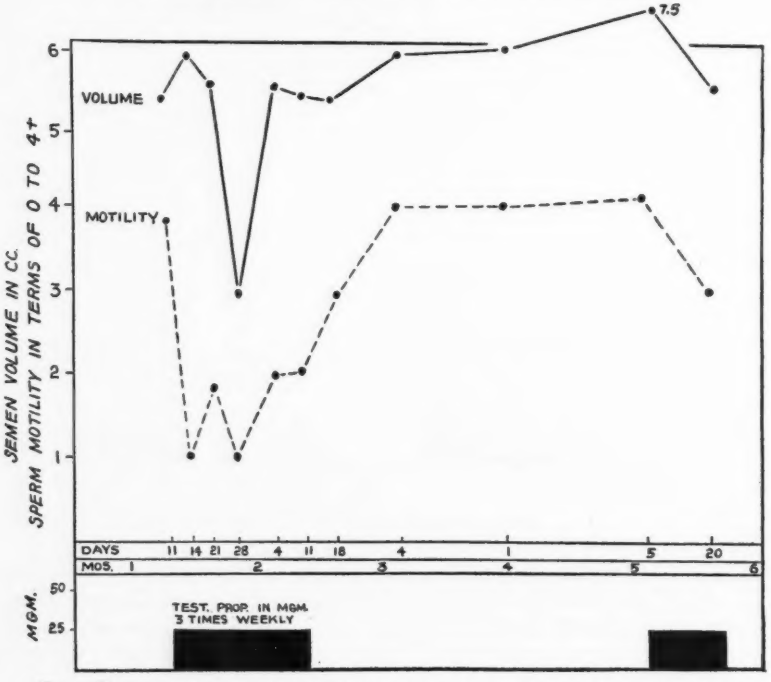


Fig. 7. The effect of testosterone propionate on the semen volume and sperm motility.

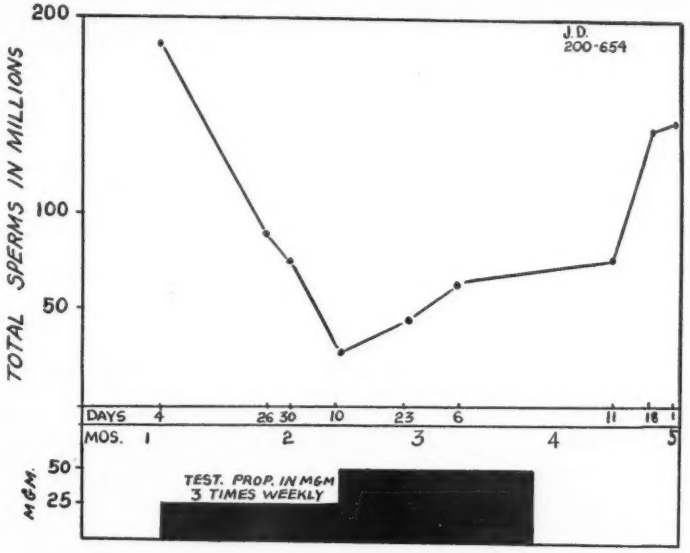


Fig. 8. Effect of testosterone propionate on the sperm count.

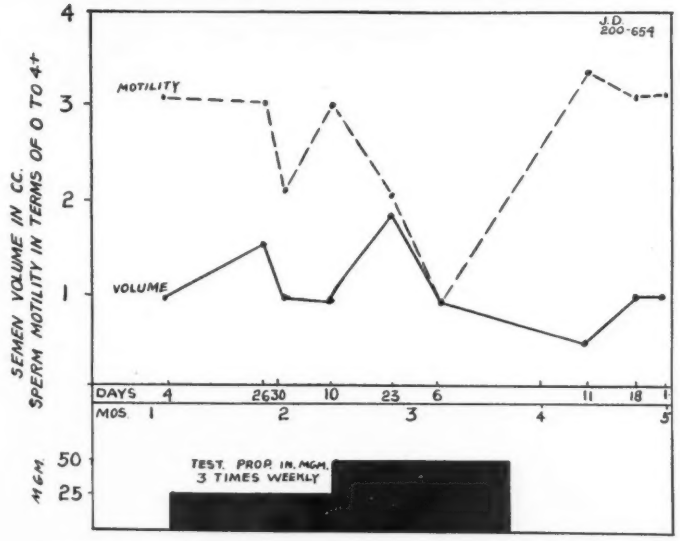


Fig. 9. Effect of testosterone propionate on semen volume and sperm motility.

for the second time. In this case pregnant mare's serum extract did not appear to increase the sperm motility nor the total number of sperm cells. Resumption of testosterone propionate therapy on January 4 resulted in decrease in sperm production, an apparent decrease in motility, and a less definite effect on semen volume. In

cases 8 and 9, the decrease in sperm count was distinct and convincing. There appeared to be a reduction in sperm motility but the suggestion of diminution in in semen volume was less definite (*case 8*, fig. 6, 7; *case* 9, fig. 8, 9).

The effect of testosterone propionate on the production of sperm cells is not always as extreme as is shown in some of the charts. One individual 20 years of age received 25 mg. intramuscularly 3 times weekly from May 11 to July 20 and still showed a total sperm count of 428 millions with good morphology and motility. Another individual 19 years of age received the same doses of testosterone propionate from April 8 to July 26, the sperm count falling from 228 millions, through various levels, to 40 millions. A third person 30 years of age received 25 mg. 3 times weekly from June 27 and on July 25 the total sperm count was 232 millions with normal morphology and motility.

DISCUSSION

Kenyon's findings regarding some of the metabolic effects of testosterone propionate have been corroborated in part.

The demonstration of an increase in the rate of epiphyseal maturity to beyond normal should serve as a warning to those who are inclined to use large doses of this material in immature individuals and suggests strongly that dwarfing might be caused by such doses given to patients who have not attained normal adult stature. Any substances which are capable of stimulating the testes to produce larger than normal quantities of androgens might be expected to produce the same effect.

The rather pronounced fall in total sperm production in the cases shown serves as evidence from human material that caution should be exercised in the use of this drug when fertility is to be preserved. It should be noted that permanent damage to sperm production has not occurred in these cases but this should not be considered as evidence that permanent damage will not follow similar therapy if it is more prolonged.

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MACROGENITOSOMIA PRECOX ASSOCIATED WITH HYPERPLASIA OF THE ANDROGENIC TISSUE OF THE ADRENAL AND DEATH FROM CORTICOADRENAL INSUFFICIENCY[1]

CASE REPORT

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THE PATIENT, *D. R. W.* (H.L.H. A. *8619*), was referred to Dr. T. Campbell Goodwin, at the Harriet Lane Home by Dr. Robert R. Harriss, of Hollywood, Florida. On admission, October 11, 1938, the boy was 3 years and 7 month. old. The father, aged 34 years, was normal except for a cardiac ailment. The mother, aged 32 years, was well except for slight irregularity of her menses. There were no evidences of any endocrine disorder in either parent, and no consanguinity. Prior to the patient's birth, the mother had given birth to a child described by Dr. Leigh F. Robinson, of Fort Lauderdale, Florida, as presenting the characteristic abnormalities of a female pseudohermaphrodite,[2] who had died at the age of 5 weeks.

During the period of gestation the mother had presented no abnormalities. The patient's birth was normal. His first teeth erupted at 5 months. He did not sit alone until the 11th month. He did not walk until the 26th month, and then it was noted that he had a stiff awkward gait. The child never learned to talk. From an early age he was very difficult to feed because of a poor appetite, and peculiar likes and dislikes of certain kinds of food. He frequently vomited when forced to eat. After his death it was learned from the mother that from the age of one year on he had shown marked craving for salt,[3] refusing to eat any food unless it was highly salted. He ate plain salt whenever he could obtain it, and was fond of saltine crackers, pretzels, potato chips, olives, pickles, salt mackerel and bacon. He disliked sweet foods. He drank large quantities of water and had polyuria and frequent enuresis. He was inclined to constipation, for which saline enemas were given every second or third day. There had been no acute illnesses.

The parents noticed at birth that his penis and scrotum were unusually large, and that his cry was deep and rough. At 5 months an acneiform eruption appeared on his face. At 15 months dark pubic hair began to grow. Several months later the mother noticed that the left testis was considerably larger than the right. He

[1] Supported by a grant from the Commonwealth Fund for the Study of the Relation of the Endocrines to Growth and Development.

[2] This term is used to describe a specific syndrome, which is discussed under "Symptomatology of Hyperplasia and of Tumor of the Androgenic Zone."

[3] The development of the unusual dietary habits in this case are discussed in a separate paper by Wilkins and Richter (in press *J.A.M.A.*). Richter has previously shown that adrenalectomized rats develop an appetite for salt. (RICHTER, C. P.: *Am. J. Physiol.* 115: 155. 1936; RICHTER, C. P., and J. F. ECKERT: *Endocrinology* 22: 214. 1938.).

never masturbated and erections were not noted. During the 6 months prior to his admission he had grown rapidly in height and strength.

Examination. The boy's height was 41½ in. (105.5 cm.), which is the average for a 4½-year-old boy. The lower skeletal segment measured 22.2 in. (56.4 cm.), the upper 19.2 in. (49.1 cm.); so that the lower extremities were relatively long in relation to the height for a child of this age. His chest was broad, with proportions of an adult male. The musculature was well developed, the subcutaneous fat scant (fig. 1). His weight was 33.5 lb. (15.1 kg.) compared to an average for his height of 37.7 lb. (16.9 kg.). The osseous development as revealed by x-rays was that of a child 10 years old. The penis, which had been circumcised, was 7 cm. long, the caliber of a man's thumb. The scrotum was large, and showed adult corrugation and stippling of the skin. Both testes were markedly enlarged for his age, the left measuring 5 × 3 cm. and the right 3.5 × 2.5 cm. They were of the normal oval shape, firmer than normal, and of a rubbery consistency. No nodules were felt in the testes. Both epididymes were readily palpable. Over the pubis there was a fairly abundant growth of long dark hair, which did not extend upward on the abdomen. There was a growth of fine blond down on the cheeks, but no axillary hair. There was an eruption of acne on the face. The voice was deep and the laryngeal cartilage was as prominent as a man's. A rectal examination was made by Dr. Vest, who made the following note: "Prostate about half normal size for a patient of 18; measures about 3 cm. wide, 1.5 cm. long, with a deep notch and furrow. Vesicles of good size, about 3.5 cm. deep, 1.5 cm. wide. Considerable secretion expressed; this, microscopically, appears to be prostatic secretion. It differs from the normal, however, in that lecithin bodies and cellular elements are rather sparse, it being mostly a turbid fluid."

Although the hair of his scalp was blond, his skin had a slightly brownish hue which was also noticeable on the buttocks and parts of the body that had not been exposed to the sun. The areolae of the nipples were quite deeply pigmented, but there were no patches of deep pigmentation about the anus or genitalia. There were no pigmented nevi or areas of vitiligo. There was a patch of brownish pigmentation on the gums over the upper incisors, but no pigmentation elsewhere on the mucous membranes of the mouth. Results of examination of the heart and lungs were negative. The blood pressure was 95/50. No abdominal masses were felt.

The child was obviously defective in mentality. He was uncooperative and expressed himself only in unintelligible sounds. His gait was somewhat unsteady and shuffling, but no definite spasticity, ataxia or tremors could be made out. Aside from this, results of the neurological examination were negative. X-ray of the skull showed no abnormalities. Ophthalmoscopic examination revealed normal eye grounds.

Laboratory data. Hemoglobin was 16.3 gm. per 100 cc. The white blood cells numbered 19,000, with the differential showing 66% polymorphonuclears, 30% lymphocytes, 3% monocytes, 1% basophils, 0% eosinophils. Intradermal tuberculin test result (0.1 mg.) was negative. Urine test results were negative, except for faint traces of albumin and an occasional pus cell. Fasting blood N.P.N. on October 14 was 99 mg.%. Blood sugar was 78.5 mg.%. Serum calcium was 11.5 mg.%. Serum inorganic phosphate was 3.2 mg.%. Plasma cholesterol was 152 mg.%. On a diet which was not meat-free a 24-hour specimen of urine contained 297 mg. of creatinine and 146 mg. of creatine.

Course in hospital. From the time of his admission on October 11 until his sudden death October 17, the boy did not seem seriously ill. He behaved like a fairly healthy but very defective child, growling and snarling in an incoherent manner when attempts were made to examine him. The idiosyncrasies of his appetite were not known at that time, and he was offered the regular ward diet. His appetite was poor and he ate but little of the food offered him. When feedings were forced he vomited on several occasions. His weight decreased from 16.95 kg. on October 11 to 14.6 kg. on October 17. The bowels were constipated, moving only with soap-suds enemas, which were given on October 13 and again on October 16. In spite of the loss of weight and the feeding difficulties, which were attributed to his mental defect and lack of adjustment to the hospital, the boy was not drowsy or obviously dehydrated. The staff felt no concern about his condition until a routine chemical examination of

his blood made on October 14 showed a N.P.N. value of 99.5 mg.%. This high value, together with the finding of a trace of albumin and a few pus cells in the urine, suggested at first the possibility of renal insufficiency, resulting perhaps from a congenital urological abnormality. On October 16 the phenolsulphonphthalein excretion was found to be 65% in 2 hours. The specific gravity of the urine ranged between 1.012 and 1.018. The blood pressure remained 95/50. On October 17 the N.P.N. had risen to 127 mg.%, the blood sugar was 78.5 mg.%, and the blood pressure was 95/45. The normal phenolsulphonphthalein excretion and the normal blood pressure seemed incompatible with a diagnosis of renal insufficiency. The high blood N.P.N., together with the dark skin and brownish pigmentation of the gums

Fig. 1. Patient *D. R. W.* at the age of 3 years 7 months.

over the incisors, suggested the possibility of adrenal insufficiency. It should have been noted, also, that there was increased concentration of the blood, as indicated by the high hemoglobin. It was decided to study the blood electrolytes.

During the morning of October 17, the boy had seemed as alert and active as ever. After eating his lunch he lay down for his nap. Shortly afterward the nurse noticed that his respirations were slow and shallow, his lips a little cyanotic, and that his right arm was twitching slightly. A few minutes later his respirations ceased entirely. Immediately after his death blood was taken by cardiac puncture for chemical studies, which showed serum sodium 111 m.eq. per liter (255 mg.%) and whole blood N.P.N. 93.5 mg.%.

Clinical impression. On admission the macrogenitosomia precox suggested a lesion in the adrenal, the testis, or the brain. The subsequent findings pointed to a corticoadrenal insuffi-

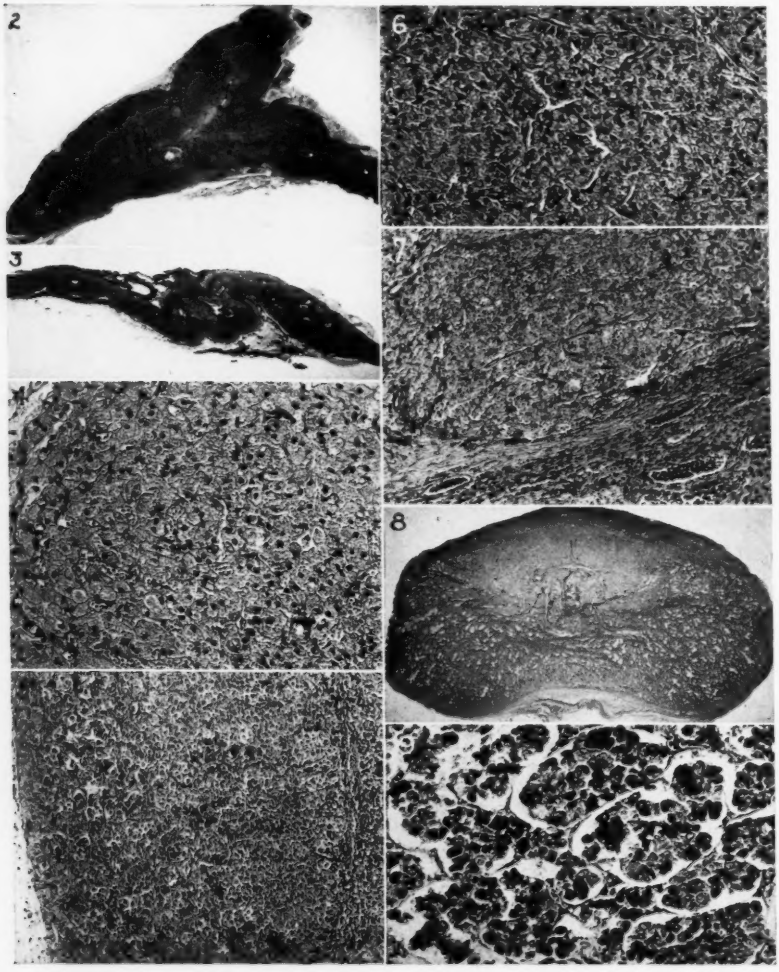

Fig. 2. ADRENAL OF PATIENT *D. R. W.* Fig. 3. ADRENAL OF NORMAL 3½-YEAR-OLD BOY (same magnification as Fig. 2) TO SHOW DEGREE OF HYPERTROPHY. Fig. 4. ADRENAL TISSUE SHOWING THE CHARACTERISTIC LARGE GRANULAR CELLS OF THE ANDROGENIC ZONE (×110). Fig. 5. ADRENAL CORTEX SHOWING ARRANGEMENT OF ZONES (×45). Fig. 6. TESTIS SHOWING ABERRANT ADRENAL TISSUE (×110). Fig. 7. TESTIS, TO SHOW REMNANTS OF COMPRESSED TUBULES (×55). Fig. 8. PROSTATE, SHOWING HYPERTROPHY OF GLANDULAR TISSUE. Fig. 9. ANTERIOR LOBE OF HYPOPHYSIS, SHOWING NUMEROUS BASOPHILIC CELLS.

ciency, and the sudden death and the low blood sodium concentration suggested an acute adrenal insufficiency, such as may occur in Addison's disease. It was by no means clear what sort of adrenal lesion could cause both a strong androgenic effect and an insufficiency of the cortical hormone which maintains the electrolyte balance.

Postmortem examination. We are grateful to Dr. W. G. MacCallum for his notes on the postmortem examinations as follows.

Anatomical diagnosis. Hyperplasia of androgenic or prenatal cells of adrenal cortex with marked diminution of normal cortical cells was found. Bilateral tumor-like masses of

aberrant adrenal cells were present in testes compressing testicular tubules. There was premature development of secondary sexual characteristics. Pigmentation of neck and shoulders (associated "Addison's disease") and acne of face were apparent.

Protocol (abbreviated). The body was the size of a child 6 to 8 years old with well developed musculature, while the features were those of a child at puberty. In addition there was a marked increase in pubic hair which was black and long and compatible with an age of 14 or 15 years. The penis was 7 cm. in length and about 1.5 cm. in diameter. Both testes were large but the left was two or three times the size of the right.

The skin over the face and neck was darkly pigmented. Over the rest of the body it was of a sallow color suggesting pigmentation. There was an acneiform eruption over the face.

All of the organs in the thoracic and abdominal cavities, apart from the adrenals and genital apparatus, appeared normal in the gross; so, too, the brain, skeleton muscular tissues and bone-marrow were normal.

The left adrenal was in the normal position but was enlarged and dark brown in color. It weighed 12 gm. On section (fig. 2) no distinct zones could be made out, but there were scattered pinpoint islands of yellow-brown tissue. The dark brown color was more evident in the outer areas but the whole structure was very dark throughout. The right adrenal was similar in appearance but even larger and weighed 15 gm.

The prostate was definitely enlarged for this age and measured 2.5 × 1.5 cm. on cross section. The seminal vesicles appeared slightly enlarged.

The left testis was greatly enlarged and weighed 30 gm. On section it had a homogeneous dark brown color, similar to the adrenals. The right testis was similar to the left but was much smaller, weighing 8 gm. There was no apparent hypertrophy of breast tissue.

The thymus was in its normal position and weighed 8 gm. It was, if anything, a little small for a child of 3 years but was otherwise not remarkable. The trachea, larynx and epiglottis were normal. The thyroid and parathyroids seemed normal.

The pineal gland was in normal position and of usual size. The hypophysis showed no gross abnormality. The brain showed no gross change.

Microscopical study. The microscopical study of the adrenals showed that they were much deformed by an accumulation of a tissue composed of large cells with vesicular nuclei and with very abundant cytoplasm which looked a little granular with ordinary stains (fig. 4, 5). With the ponceau-fuchsin stain such cells showed quite brilliantly in many places the deep red staining which is thought by Broster and Vines (1) to be characteristic of the androgenic type. The masses of these cells distorted the adrenals in places while other portions more closely approached the usual arrangement of cortical layers. These same large cells filled most of the fasciculate layer. The medulla of the adrenal appeared normal.

Sections through the testis on both sides showed an almost complete displacement of the normal tissue of the testis. The remaining tubules were found practically only in the surrounding capsule where they were greatly compressed. The epididymis was not altered. The masses which caused this displacement (fig. 6, 7) were similar to those described in the adrenals—made up of the same type of cell which stained in the same way with the Vines stain. In a careful search no Leydig cells were seen, and it appears far more probable that these masses, which are so like those found in the adrenal, were derived from aberrant tissue. This is supported by the fact that in the abdominal cavity there were found in the neighborhood of the kidney at least two small nodules which on section proved to be composed of the same cells as were present in the adrenal.

The prostate was enlarged, as described, and showed an over-development of the glandular tissue (fig. 8). The thyroid and parathyroid showed no special microscopical alterations. The hypophysis in general appeared normal except that there was a distinct relative predominance of basophilic cells scattered throughout the anterior lobe (fig. 9). Sections of the brain through pons, midbrain and cortex showed no abnormalities.

Bioassays of urine, testes and adrenals. Assay of the urine for estrogenic substances showed less than 10.5 R.U. per liter, or less than 8 R.U. per 24 hours by the method of Smith and Smith (2).

The assays for androgens were carried out according to the castrate rat colchicine method of Fleischmann (3) as follows. The benzene extract of urine after evaporation was taken up in olive oil. Half of this was injected on each of 2 successive days into castrated male rats. Nine hours before killing the rats, they were injected with 0.2 mg. of colchicine per 100 gm. of body weight. The finding of numerous mitoses in the epithelium of the seminal vesicles is considered a positive test, which is brought about by 5 I.U. of androgen. This method showed that the urine of the patient contained 66 I.U. per liter, or 30 I.U. for that particular day. A control test run with the urine of a normal man showed an output of 20 I.U. per liter, or 30 I.U. per day.

The adrenals and testes of the patient were extracted with 95% alcohol according to the method of Moore (4). The alcoholic extract after evaporation was dissolved in benzene, then in acetone and finally in olive oil. Assayed by the castrate rat colchicine method the extract from 6.3 gm. of the patient's testes gave a yield of 5 I.U. of androgen, whereas the extract from 31 gm. of testes of a normal 38-year-old man failed to show androgen. A similar extract from 2 gm. of the patient's testes, assayed by the method of Frank and Klempner (5), caused growth when rubbed into the comb of a 11-day-old-chick over a period of 7 days. (The treated comb weighed 39 mg., whereas the comb of a control chick of the same age weighed 14 mg.)

The chick-comb assay of the extract from 1.4 gm. of the patient's adrenals gave a positive test (weight of treated comb 20 mg., control 6 mg.). The extract from 3.5 gm. of adrenals by the castrate rat colchicine method failed to show measurable androgen. (No larger amount of the material was available for assay.)

SUMMARY OF ANDROGEN ASSAYS[4]

Urine: 66 I.U. per liter, 30 I.U. per day } castrate rat colchicine method
Testes: 6.3 gm. contained 5 I.U.; castrate rat colchicine method
2 gm. contained 1 I.U.; chick-comb method
Adrenal: 1.4 gm. contained 1 I.U.; chick-comb method
3.5 gm. negative; castrate rat colchicine method

The Difference in the Function of the Androgenic Zone Cells and the Interrenal Cells

The prenatal zone of the adrenal and its involution after birth were described by several investigators in 1911 (6, 7, 8). In the human fetus this zone forms the main bulk of adrenal glands, which are relatively large. Soon after birth it underoges a rapid involution, which is almost complete by the end of the first year (9). After the third year, it is represented merely by a narrow layer of connective tissue and a few cells in the innermost portion of the adrenal cortex (10). E. Howard (11) found a similar zone in the mouse, which persists until adolescence. According to her observations, castration causes a prolonged persistence of the zone in the male, but is without effect in the female. Gradually clinical and pathological evidence has accumulated, which suggests that the enlargement of the adrenals which occurs in the female pseudohermaphrodite is caused by hyperplasia of the cells of this zone, and that the virilizing tumors of the female contain the same tissue (1, 12, 13). The marked difference in function between these cells and the other portions of the adrenal cortex which control the electrolyte balance was first clearly emphasized by Grollman (14), who called them the "androgenic zone" and the electrolyte-controlling portions of the cortex the "interrenal cells."

[4] It is realized that these assays are only approximations, due to the fact that so few test animals were employed. The small amounts of tissue and of urine available made it impossible to use a larger number.

Until now no cases have been reported[5] which offered substantial proof that a hyperplasia of the androgenic zone (causing virilizing effects) might lead to a destruction of other portions of the adrenal cortex producing adrenal insufficiency of an Addisonian type. However, a number of cases in the literature are suggestive of this. Gallais (15) reported a girl with adrenogenital syndrome who showed muscular weakness and pigmentation "like a panther." Priesel (16) mentions a "strong pigmentation of the skin" in the case of a 5-year-old girl with pseudohermaphroditism due to adrenal hyperplasia. This child had a brother (reported also by Orel, 17) with macrogenitosomia who also showed brown pigmentations and died at 2½ years in convulsions. No autopsy was performed. Hoag (18) in a review of 22 cases of malignant hypernephroma having virilizing effects in children, stated that a few showed distinct pigmentation of the skin. Macera (19) reported a boy with hypertrichosis and macrogenitosomia due to a malignant hypernephroma, who died at 2½ years in convulsions. Grollman (14) stated "certain other clinical symptoms described as associated with adrenal virilism are nausea, vomiting, weakness and rarely pigmentations on the forehead and border of the axilla. These are symptoms of Addison's disease and are due to the encroachment of the malignant androgenic zone on the true cortical tissue." In our case the suggestive pigmentation of the skin and gums, the craving for salt, the low blood sodium, the high blood N.P.N. and the sudden death are clear evidences of a corticoadrenal insufficiency which was explained at autopsy by the almost complete replacement of the normal cortical structure by the hyperplastic androgenic zone. It therefore confirms the theory of the difference in the function of the two tissues.

Microscopically, the differentiation of the androgenic cells from the interrenal cells of the cortex is not entirely satisfactory. Attempts have been made to find a specific stain for the androgenic zone. In the hands of Broster and Vines (1) the ponceau-xylidine-fuchsin stain apparently has been satisfactory. A stain composed of Poirier blue and eosin seems to stain the androgenic cells deep purple, more or less specifically, but none of these stains seem quite satisfactory as yet.

Comparatively little is known concerning the nature and mode of action of the hormones produced by the androgenic zone of the adrenal. Reichstein (20) has isolated a substance from the adrenals of cattle, which differs from the cortin-like fractions. It is a tri-ketone which shows about one-fifth the activity of androsterone in the chicken comb test, and has no estrogenic activity. From the same source Reichstein also isolated (20) another compound, 11-oxy-trans-androsterone, which has one-thirtieth the androgenic activity of androsterone (21). Friedgood and Gargill (22) have demonstrated the presence of androgens in a malignant adrenal cortical tumor in a 10-year-old girl by a colorimetric method. The benzine-soluble fraction contained 8.5 I.U. of androgen.

In the urine of this and other patients with adrenal tumors, androgens have been found frequently in large amounts. Burrows, Cook, Roe and Warren (23) found in the urine of a man with adrenal tumor, a very high estrogen content with a moderately high androgen content. They isolated from the urine the compound androstadien-17-one, closely related to dehydroandrosterone. In the urine of a 7-year-old girl suffering from adrenal tumor, Callow (24) found 3500 I.U. of androgen per liter at one time, and 500 I.U. at another time. Seventy per cent of the androgenic activity could be accounted for by dehydroandrosterone, which was isolated in pure crystalline

[5] Since this article was written, Butler, Ross and Talbot have reported the case of a boy of 20 months who showed macrogenitosomia, hyperpigmentation of the skin and corticoadrenal insufficiency. Although pathological studies have not been made he probably presents the same syndrome as our patient. *J. Pediat.* 15: 831. 1939.

form. Callow also found in the urine of a 19-year-old girl with adrenal tumor an androgen excretion of 320 I.U. per liter. Butler and Marrian (25) have isolated isoandrosterone from urine obtained from a woman with an adrenal tumor and marked symptoms of virilism. Koch (26) found the androgen excretion in 4 cases of virilism to be respectively 0, 44, 69 and 480 I.U. per day. Bruins Slot (27) found 2200 I.U. of androgen per liter in the urine of a woman suffering from virilism due to suprarenal tumor, but was unable to demonstrate androgen in the tumor itself. The assay in this case was made by Dingemanse and Laqueur (28), who have found excessive amounts of urinary androgens in two other cases of adrenal tumor. Twelve hundred I.U. of androgens and 13,000 I.U. of estrogens per liter were found in one case of a woman with virilism, 600 I.U. of androgens and 1000 I.U. of estrogen in the case of a man with adrenal tumor presenting the symptoms of Cushing's syndrome. Friedgood and Whidden (29) found the androgen excretion in 2 cases of virilism due to adrenocortical tumor to be the equivalent of 45 and 325 mg. androsterone per 24 hours at various stages of the disease. In one of these cases mentioned previously, they demonstrated androgen in the tumor tissue. The androgen excretion fell to normal levels after removal of the tumor, but began to increase again about 4 months before clinical evidence of recurrence was detected.

In the case which we are reporting, the extract from 1.4 gm. of the adrenal tissue showed 1 I.U. of androgenic activity by the chick-comb method. (The urine showed as much androgen per day by the castrate rat colchicine-method as that of a normal man.) The extract of the testes, which apparently were composed almost entirely of the androgenic zone cells, showed approximately the same androgen content as the adrenals themselves. By the castrate rat colchicine test 6.3 gm. of testis show 5 I.U. of androgen, and 2 gm. of testis showed 1 I.U. by the chick-comb method. A similar assay by the castrate rat colchicine method carried out on the extract from 31 gm. of testes of a normal 38-year-old man gave negative test results.

That the androgens cannot substitute for cortin is borne out also by the experiments of Spurr and Kochakian (30), who showed that androstenedione, testosterone acetate and testosterone propionate do not prolong the survival time in adrenalectomized rats.

It seems probable that the adrenal androgens act directly on the accessory male sex organs and not through the intermediary of the interstitial cells of the testes. In cases of virilization of the female no tissue is found corresponding to the Leydig cells. In our case, the interstitial cells were entirely absent. In most of the cases of macrogenitosomia precox in boys resulting from adrenal tumors the testes have been immature, the Leydig cells have not been increased and spermatogenesis has been absent.

The Occurrence of Adrenal Cells in the Testes

For the explanation of the presence of adrenal cells in the testes of our patient, one must turn to embryology. In the embryo of about 6 mm., or the fourth week of development, the gonads and the adrenal glands begin to develop from the mesothelial cells lining the coelomic cavity. Both arise from the same vicinity on the genital ridge. At 6 to 7 weeks the cells which form the gonad begin to differentiate into either the testes or the ovary. The further details of the differentiation of the testes into the interstitial cells and the germinal tubules, and of the adrenals into androgenic zone and interrenal cells, are still unknown. The close relationship of the gonad and the adrenal make it quite possible that the adrenal cells may become included in the developing testes, or that a common precursor or anlage of the cells which give rise to both the gonad and the adrenal may undergo an uncontrolled hyperplasia in

both organs. It is known that cells of the type of the androgenic zone migrate also to other parts of the abdominal cavity, giving rise to accessary adrenals. These were described as long ago as 1883 by Marchand (31). They occur beneath the lower pole of the kidney, on the iliopsoas muscle, along the spermatic cord between the testis and epididymis, and in the rete testis and paradidymis (32). They are found on the fallopian tubes, in the broad ligament and in the ovaries in a large percentage of all new-born females. After the period of life when the androgenic zone of the adrenal disappears, these accessory adrenal bodies are not found at autopsies of normal individuals. However, in cases of female pseudohermaphroditism accessory adrenals are frequently found, and in later life tumors arising in these structures may cause virilization of the female (33).

The presence of the adrenal cells in the testes of our patient is evidence that the abnormality arose in early embryonic life. Although no Leydig cells could be identified, one cannot be sure that they were entirely absent. The absence of seminiferous tubules, except for a small vestige, probably was due to their crowding out and destruction by the overgrowth of adrenal tissue rather than to a hormonal influence.

Symptomatology of Hyperplasia and of Tumor of the Androgenic Zone

In the female. Hyperplasia of the androgenic zone of the adrenals occurring in the young female embryo gives rise to certain abnormalities which are characteristic of female pseudohermaphroditism. The clitoris is enlarged, the urethra opens just beneath this organ as in hypospadias, and there is no external vaginal orifice present. At a short distance from the meatus a rudimentary vagina opens into the urethra. At the neck of the bladder, there is a well developed prostate made up of the lateral lobes but lacking in the posterior lobe. Infantile uterus, fallopian tubes and ovaries are present. In some instances pubescence with the development of adult male characteristics occurs as early as the second year, whereas in other cases it is delayed until the normal time. Why this difference occurs is not known. Young, in his monograph, gives an excellent discussion of the way these abnormalities are brought about by an overdevelopment of the Wolffian duct system and a suppression of the development of the Mullerian ducts. He has collected 23 cases from the literature and has reported 7 cases of his own. In all, there are probably about 40 cases of the condition now on record.

As emphasized by Howard (13), tumors of the adrenal or hyperplasia occurring in postnatal life do not cause the characteristic anatomical abnormalities of female pseudohermaphroditism but may lead in the female to virilization and precocious puberty.

In the male. Eleven cases have been reported (19, 34–43) in which adrenal tumors have caused premature sexual development in boys. The tumors are frequently malignant, metastases to other organs occurring in 6 of the 11 cases. The symptoms mentioned were growth of the penis to adult size, well developed prostate, pubic and facial hair, acne and a deep voice. There was excessive growth and muscular development, but frequently mental deficiency. The first symptom of premature sexual development usually noted was the appearance of pubic hair which occurred between the ages of 6 months and 18 months in 8 cases, and at 4 years, 10 years and 11 years in the other cases. In spite of the early appearance of the secondary sexual characteristics, the testes were described as either normal or hypoplastic in all but one case (19), in which they were large. Fordyce and Evans (40) noted that Leydig cells were very scanty. In no instance was spermatogenesis found. In only one case, that of a 5-year-old boy (42), were nocturnal emissions and erections reported. This is in

marked contrast to the cases of true sexual precocity following brain lesions. In some of these cases marked sexual desire and spermatogenesis have been demonstrated.

Our case is the first in which at autopsy a diffuse bilateral hyperplasia of the adrenals has been found in the male, causing a condition analogous to pseudohermaphroditism in the female. There are 2 cases in the literature which are suggestive of the same condition, but autopsies were not performed. Orel (17) and later Priesel (16) described a boy of 28 months with well marked sexual precocity and dark pigmentation of the skin. His testes were small and situated in the inguinal canals. One sibling had the characteristic abnormalities of female pseudohermaphroditism and showed brownish pigmentation of the skin. At the age of 5½ years she died in convulsions during the course of scarlet fever. Hyperplasia of the adrenals was found at autopsy. The boy died in convulsions at 28 months, but unfortunately an autopsy was not obtained. No tumor of the adrenals had been felt. Jacobziner and Gorfinkel (44) in 1936 reported an interesting family. A boy of 6½ years developed pubic hair at 18 months and presented all the characteristics of our patient, except the pigmentation of the skin. The testes were said to be "the size of a normal adult's." Two sisters, 5 years and 2 years old, were pseudohermaphrodites. In none of the children was any abdominal mass felt. Exploratory operations were not performed.

The Cause of Hyperplasia of the Androgenic Zone

The cause of the hyperplasia of the androgenic zone which begins in embryonic life and persists postnatally is not known. The question arises whether it is brought about by the influence of the pituitary or other endocrine glands. In our case there was an increase of the basophilic cells of the pituitary, but no tumor of the pituitary was found, nor any lesion in the brain. It seems probable that pituitary basophilism might be a result rather than the cause of the adrenal hyperplasia.

The familial occurrence of adrenal hyperplasia deserves special emphasis. Our case is the eleventh in which multiple instances have occurred in the same family. Hermaphroditic sisters have been reported in two families (12), and in one family each by others (1, 45–48). Priesel (16) reported a female pseudohermaphrodite who had a brother with sexual precocity, and Jacobziner and Gorfinkel (44) found a sexually precocious boy and 2 pseudohermaphroditic sisters in the same family. O'Farrell (49) reported that there were 5 hermaphrodites out of 40 children born to 6 members of the same family, but it is not certain from his description that the hermaphroditism was due to adrenal hyperplasia.[6] In these families the sexual abnormalities have occurred in only one generation. In no instance did either parent present definite evidence of any endocrine disturbance. Therefore, there is no reason to suppose that the adrenal hyperplasia in the embryo is brought about by a hormonal unbalance in the mother.

SUMMARY

A case is reported of a 3½-year-old boy who had precocious development of the male accessory sex organs. A sibling was a female pseudohermaphrodite. The patient had moderate pigmentation of the skin and gums, and a low blood sodium, high N.P.N. and a craving for salt. He died suddenly. The adrenal cortex was composed largely of androgenic or prenatal zone cells, which apparently replaced other elements of the cortex. The testes were large and composed of similar cells. No spermatogenic cells were found. To our knowledge, this is the first case in which there is substantial proof that death from adrenal insufficiency resulted from the destruction

[6] *Case* 3 reported by O'Farrell has recently been explored by Dr. Hugh H. Young, who found both adrenals were much enlarged.

of the electrolyte-controlling elements of the adrenals by hyperplasia of the androgenic zone. It supports the theory that the androgenic zone has a function different from the portions of the cortex which control the electrolyte balance. Macrogenitosomia precox has been reported in the male with adrenal tumors. In the case presented it was due to an embryonic hyperplasia of the androgenic zone, and is therefore analogous to the embryonic virilization which causes pseudohermaphroditism in the female. The familial incidence of such types of sexual abnormality is of interest, and has been reported previously in the literature.

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MASCULINIZING AND NON-MASCULINIZING CARCINOMATA OF THE CORTEX OF THE ADRENAL GLAND

REPORT OF SIX ADULT CASES

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THE CLINICAL PICTURE associated with neoplasm of the cortex of the adrenal glands may vary as widely as the physiologic processes in which that structure plays a part. Cushing's basophilism, masculinization, feminization, Addison's disease, simple malignant cachexia and renal syndromes, may each or all result from new growths, the histologic features of which are not strikingly or gratifyingly distinctive. Experimental work on normal animals has far outdistanced clinical analysis and conclusion. It is with the hope of adding more clinical data for which logical associations can be made that 3 cases of cancer of the cortex of the adrenal gland, with masculinization, and 3 without secondary sexual changes are reported.

Carcinomata with Masculinization

Case 1. E. T., a 34-year-old white female, 5 ft., 3 in. tall, was admitted to the hospital complaining of weakness, dyspnea on exertion, and slight ankle edema during the preceding year. She had always been fat, but during the preceding 5 months had gained from 190 to 245 lb., and had noticed increasing polyuria and polydypsia. Her past illnesses included typhoid fever, measles, chicken pox and syphilis (adequately treated). Her menses began at the age of 9, and until 6 years ago, occurred every 28 to 30 days and lasted 4 days with "good flow." Amenorrhea of 3 months duration occurred at the age of 28, and again for 12 months at 32. The flow was reestablished for 4 months during her 33rd year, and had been absent for 6 months at the time of admission to the hospital. Her family history was essentially unimportant. Noteworthy in her physical examination were the short, stocky build with moderate upper dorsal kyphosis; moon shaped, but not plethoric, face; excessive fat about the neck, shoulders and abdomen with comparatively thin legs and arms and small hands and feet. Her skin was dry; there were a few non-pigmented striae over the abdomen. There was some hairiness of the chin and upper lip, a very moderate increase in hair over the legs and arms, and a male type of abdominal escutcheon. Her visual fields and fundi were normal. The heart was slightly enlarged; the rate 108; the blood pressure 180/95. The external genitals were atrophic; the uterus small and ovaries apparently normal.

Laboratory data (table 1) included: normal results of urinalysis except for traces of sugar; a repeatedly negative blood Wassermann and a normal sedimentation rate for erythrocytes. Her blood count showed red cells, 6.84 millions; hemoglobin, 118%; white blood cells 5,700, with a normal differential count. The B.M.R. was +15%. Other blood chemical constituents in mg.% were: N.P.N. 30.0; creatinine, 1.5; glucose, 154; calcium, 9.0. Blood plasma proteins were 5.8%; the CO_2 combining power of the blood plasma 75 volume %. Hourly readings of blood sugar after ingestion of 100 gm. of glucose were 200, 308, 364 and 400 mg.%. X-rays of the spine, arms and legs revealed moderate osteoporosis. Skull plates showed a sella turcica of normal size. Retrograde pyelography demonstrated a downward displacement of the left kidney.

The patient's course in the hospital was rapidly downhill and terminated with the

picture of bronchopneumonia, more marked on the right side, on the twelfth day after admission.

The essential postmortem findings included a grossly and microscopically normal pituitary gland (a Crooke's count was not made). The right adrenal gland was normal. The left adrenal gland weighed 220 gm. and contained a tumor 8 × 6 × 5 cm. in size, which showed areas of hemorrhage and coagulation necrosis. A stained section showed many small and moderately sized sinus-like blood vessels surrounded by large granular and fatty cells. Giant cells of large size were scattered throughout the structure. The neoplasm had invaded the left suprarenal and renal veins and metastases were present in the retro-peritoneal lymph nodes and in the lower lobe of the right lung. The postero-lateral bronchus of the latter was completely occluded by a mass, the histologic features of which were identical with the

TABLE 1. LABORATORY FINDINGS IN CASES 1 TO 6

		Non-masculinizing			Basophilic	Masculinizing	
	Normal	*Case 6,* S. W. (F) (2 wk. before death)	*Case 5,* A. T. (M)	*Case 4,* G. M. (F)	*Case 1,* E. T. (F)	*Case 3,* A. M. (F)	*Case 2,* C. B. (F)
B.M.R.	−10 to +15	+2	+1		+15	+41	−5
Red blood cells	4.3–5.2	3.32	4.47		6.84	3.50	3.0
Hemoglobin	80–90	63	—		118	72	70
Plasma protein	6.5	7.3	6.3		5.8	6.3	4.6
Blood volume	4400 cc. F. 5200 cc. M.	4450 cc.					
N.P.N.	35.0	—	24.0		30.0	28.0	
Urea	15–20	15.1	—	65.3	—		37
Creatinine	1.0–2.0	1.6	1.3	4.5	1.5	1.4	
Sugar	80–120	153	103	90	154	107	53
Cholesterol	100–190	1590				190	95
Esters							50
Sodium	330	341.0		261.3		293.8	
Potassium	16–20	17.2					
Chlorides	355–385	412				312.7	289.5
Calcium	9.0–11.0	10.9			9.0	10.0	10.5
CO_2 combining power	65	42			75		
Glucose tolerance		Normal			Low	High	High
Phosphorus						3.12	

primary growth. The ovaries were slightly smaller than normal and showed some cystic changes. Clinical diagnosis was Cushing's syndrome secondary to carcinoma of the cortex of the left adrenal gland. Anatomical diagnosis was carcinoma of the cortex of the left adrenal gland with metastases to the retroperitoneal lymph nodes and to the right lung; terminal bronchopneumonia.

The essential lesion in pituitary basophilism seems still to be a mystery. Of Cushing's (1) originally reported 16 cases, only 11 had complete autopsy, and of these only 4 showed no other endocrine lesion than a basophilic adenoma of the pituitary gland. Hare, Ross and Crooke (2) using Rasmussen's (3) technic believed that the syndrome arises from hyaline and other changes in the non-adenomatous basophils. On the other hand, Kepler (4) favored the adrenal in a basic rôle with the pituitary disturbances occurring secondarily. The problem of etiology becomes all the more confusing when it is realized that the syndrome has been described without demonstrable endocrine lesion anywhere (5, 6), and in the course of thymic carcinoma (7, 8). Numerous instances of the syndrome in connection with adrenal cortical carcinoma are now on record (8–17). In Graef's case (15) a microscopic adenoma of

the pituitary gland was also found. The present case emphasizes the electrolytic changes which characterize basophilism. Hyperactivity of the adrenal cortex is suggested by the contrast between the results seen here and those noted in Addison's disease. Others have made similar observations (18, 19, 20).

The osteoporosis may be explained on the basis of increased calcium excretion or of failure in calcium and phosphorus absorption, both of which are stated to occur in cases of Cushing's syndrome (19).

Case 2. C. B., a 29-year-old white married female, 5 ft. 6 in. tall, and weighing 118 lb. was admitted to the hospital on 2/13/35, complaining of loss of weight, weakness, pains in the legs, amenorrhea and hirsutism. She had been perfectly well until 2 years before when her periods, which had been established at the age of 14, had previously occurred at 30-day intervals and lasted 5 days, became irregular. One year prior to admission, following a "pneumonia" they ceased entirely. Since that time she has lost approximately 50 lb., has observed gradually increasing hirsutism, anorexia, weakness, pigmentation of the extensor surfaces of the hands and forearms, and pains in the extremities, particularly marked on the left.

Physical examination revealed a somewhat cachectic, chronically ill female with pallid skin save for the areas of pigmentation already mentioned. The tongue was atrophic at the tip. The thorax was symmetrical; the lungs clear. The heart was not enlarged; the blood pressure, 82/50. The abdomen was soft; an irregular firm liver edge could be felt 3 fingers below the right costal margin. In the left hypochondrium a mass "about the size of a grapefruit" could be made out. Vaginal examination revealed no changes in the external genitalia or uterus; the ovaries were not palpated.

Laboratory studies (table 1) revealed a urine of fixed specific gravity (1.008); an icteric index of 12; a positive delayed direct van den Bergh, a negative blood Wassermann; a sedimentation rate of 15 mm. in 15 minutes and 133 mm. in 1 hour. The B.M.R. was −5%. There were 3.0 million erythrocytes and 7350 leucocytes per cu. mm.; the hemoglobin was 70%. Blood plasma proteins were 4.6%. Other blood constituents in mg.% were: urea nitrogen, 37; sugar, 53; total cholesterol, 95, esters, 50, chlorides, 289.5, calcium, 10.5. The glucose tolerance curve with hourly determinations showed 92, 108, 124, 88 mg.%, respectively. Retrograde pyelograms revealed a normal right kidney. The left kidney was pushed downward, its upper pole rotated medially, and its pelvis anteriorly.

Operative procedure was undertaken on 2/21/39, through a posterior incision, and a large mass discovered in the left suprarenal region, which was so adherent to surrounding structures as to make removal impossible. A section removed for biopsy revealed a highly anaplastic carcinoma of the adrenal cortex. The patient's post-operative course was rapidly downhill to death on the third day following operation. Autopsy was refused. Clinical diagnosis was carcinoma of the cortex of the left adrenal gland with metastases to the liver, and possibly to the lung; secondary adrenal insufficiency.

The clinical picture in this case suggests the simultaneous presence of masculinization, malignant cachexia and Addison's disease. Cahill (21) described a wide variety of clinical changes in his cases of tumor, including, in some instances, pigmentation and "metabolic changes of plasma fat and skin." Loeb (22) observed wide fluctuations in the blood electrolytes in his cases with Cushing's syndrome. It is possible that in these instances he was faced with varying degrees of adrenal insufficiency, superimposed upon a preceding overactivity.

Case 3. A. M., a female aged 38, whose condition has been reported in detail elsewhere (22) had developed diminution in menstrual flow when 36 years old, and complete cessation of her periods 9 months before coming under observation. Associated were loss of libido, deepening of the voice, generalized hirsutism tendency to male habitus with angular shoulders, slim hips and atrophic breasts, progressive loss of weight, increasing abdominal girth and generalized weakness. Two months prior to hospitalization cough, hemoptysis, chest pain, dyspnea, right pleural effusion and fever appeared.

Her general appearance was cachectic. There were signs of fluid in the right chest with atelectatic lung above. The breasts were atrophic and showed hair about the areolae. The blood pressure was 130/80. A large mass, not separable from the liver, occupied the entire upper abdomen with its lower edge opposite the iliac crest on the right, and sloping upward to a point just above the umbilicus on the left. There was a male escutcheon, an enlarged clitoris and bilaterally cystic ovaries.

The blood showed a secondary anemia with an absolute and relative polymorphonucleocytosis. The blood chemical constituents are as noted in table 1. Results of the Wassermann and van den Bergh were negative. The sedimentation rate was markedly increased; the B.M.R., +41. An increased tolerance was exhibited for glucose. The bitterling test was normal. Between 1000 and 2000 mouse units of estrin were found per liter of urine Retrograde pyelography showed characteristic displacement of the right kidney. Other laboratory findings were essentially negative. Six weeks prior to death she expectorated a neoplastic mass 3 × 2 × 2 cm. Her death occurred from asphyxia due to endobronchial extension of a metastatic growth.

Complete autopsy, except of the head, was performed while the body was still warm. The primary carcinomatous growth in the right adrenal gland measured 21 × 17 × 12 cm., and weighed 1990 gm. The liver, including a metastatic nodule 17 × 15 × 12 cm., replacing nine-tenths of the cystic lobe, weighed 4540 gm. Smaller nodules were present throughout its substance. Near the hilus of the right lung, and extending intrabronchially was a 2.5 × 2.4 × 2.1 cm. mass. Many smaller nodules were seen in the parenchyma of both lungs, and on the surface of the pleurae. Sections of the original tumor and of its metastases, showed areas of hemorrhage and coagulation necrosis interspersed with characteristic carcinomatous cells. The Broster Vines reaction on the adrenal, hepatic and pulmonary new growths was negative.

The anatomic diagnoses included massive masculinizing primary carcinoma of the cortex of the adrenal gland with extensive secondary coagulative and lytic necrosis, hemorrhage, and apparently complete destruction of all normal glandular tissue; moderate atrophy of the left adrenal gland; metastases to the liver, lungs and pleurae; complete occlusion of the right primary bronchus, partial atelectasis of the right lung, right sided bronchopneumonia, bronchiectasis, moderate right venous thrombosis, and pulmonary fibrosis,—all secondary to metastatic neoplasm; massive ascites; bilateral ovarian atrophy and uterine leiomyomata.

This appears to be the second largest, if not the largest, adrenal carcinoma ever found in an adult. A 34-year-old woman described by Walters, Wilder and Kepler (8) showed a lesion "approximately 25 cm. in diameter," but the weight is not given. A 13-year-old female, reported by Kennedy and Lister (23) showed a tumor measuring 21 × 12.5 × 6 cm. and weighing 1860 gm. Van der Bergh (24) saw an adrenal carcinoma 22 × 16 × 12 cm. of 2000 gm. mass in a girl 3 years of age.

The size of hepatic metastasis and the expectorated mass are also noteworthy. Burke (25) mentions a nodule in the liver, 8 cm. in diameter. Apparently dislodgement of an endobronchial mass 3 × 2 × 2 cm. has not been previously reported in the course of an adrenal carcinoma.

The specificity of the fuchsinophile reaction in masculinizing lesions of the adrenal cortex has been widely discussed (2, 21, 26–29). The vividly red staining of the inner layers of the cortex of adrenal glands removed for virilism was first described by Vines (30). He found a positive reaction to a ponceau fuchsin dye in 34 of 36 glands removed for the relief of the symptoms of masculinization (26). Further studies with the stain showed that it is also taken up with avidity by the acidophilic cells of the pituitary, the interstitial cells of the testis and the young corpus luteum. Moreover, while the reaction is commonly absent after birth, it is normally positive in both the male and female glands during certain developmental stages of fetal life. The greatest activity is seen during the weeks of sex differentiation and the development of the pituitary into a solid gland. This period lasts longer in the male than in the female.

It is the belief of the majority of workers that the adrenal gland exerts a strong "bisexual" function at this time. This does not completely disappear in later life, as shown by the presence of male sex hormone in the normal adult female and vice versa. In disturbances such as virilism the hormone of the opposite sex is seen in excessive amounts. Broster (31) states that "what evidence there is points to the fact that the adrenal cortex is the main source of the excess hormones found."

If these conceptions are correct, and they seem to be well supported by fact, the absence of the ponceau fuchsin reaction in the adrenal tumor and its hepatic and pulmonary metastases is not easy to explain in the present instance. The time elapsing between death and fixation of tissue may be a factor. Vines (26) claims that the staining contrast is less marked the longer the interval, whereas Cahill (27) states that the ability of the tissue to respond becomes more noticeable as post-mortem changes proceed. If the latter conception proves to be the correct one, sufficient time may not have elapsed in the present instance to allow such alterations to take place.

It seems more plausible, however, to suggest that inasmuch as the masculinization of this individual reached its acme at least 8 months prior to death, secondary pressure, hemorrhagic and necrotic changes destroyed the majority of the cells which were functioning in a "bisexual" manner. Further consideration of the reaction is being undertaken in the light of these findings.

Carcinomata without Masculinization

Case 4. G. M., a 70-year-old white female, was admitted to the hospital on 4/10/34 with complaints of constipation, increasing to obstipation, during 2½ years; pain in the left lumbar region for 2½ years; a left upper abdominal mass first noted 1½ years ago; generalized weakness and loss of weight (60 lb.) throughout 2 years. Her difficulties had begun with the pain, which was usually so severe as to be relieved only by large doses of opiates. The patient was a pale, cachectic individual with dry wrinkled skin, but no pigmentation. All observable arteries showed advanced arteriosclerosis. The tongue was dry and thickly coated. Her heart and lungs were normal. The blood pressure was 100/60. Urinalysis on several occasions revealed a faint trace of albumin, many hyaline and granular casts, and many red blood cells. The Vollhard's concentration test showed a fixation of specific gravity between 1.018 and 1.020. Results of the blood Wassermann and the van den Bergh tests were negative. Blood urea nitrogen, one month before death, was 65.3 mg.% and creatinine 4.5 mg.%. The icteric index was 5. The stool showed no mucus, pus or occult blood. In x-ray studies of the gastro-intestinal tract, the descending colon appeared to be pushed forward, downward and lateralward.

Retrograde pyelography revealed diminished excretory power on the part of both kidneys, and demonstrated downward and forward displacement of the left kidney with incomplete filling of its pelvis.

The patient's course was progressively downhill, with gradually increasing uremia, to death on 7/29/34. On postmortem examination, there was a large mass 15 × 9 × 5 cm. in the left suprarenal region which was firmly attached to the capsule of the kidney but did not penetrate its substance. Widespread adhesions to the intestines and the peritoneum were present. The tumor mass had invaded the suprarenal and renal veins, causing thrombosis. Metastases were present in the retroperitoneal lymph nodes, the liver and the lungs. The brain and long bones were not examined. The right adrenal was atrophic and weighed 6 gm. Sections through the tumor revealed a lobulated appearance with widespread hemorrhages and areas of necrosis. On microscopic study, areas of degeneration and tumor growth were present. The cells were large, frequently vacuolated with many mitotic figures. Both kidneys were small, with narrowed distorted cortices, and presented the microscopic picture of chronic diffuse nephritis.

The anatomical diagnoses were carcinoma of the cortex of the left adrenal gland with

metastases to the retroperitoneal lymph nodes, the peritoneum, the liver and the lungs; atrophy of the right adrenal gland; chronic diffuse nephritis with chronic uremia; generalized arteriosclerosis.

Cahill (21) remarks that pain in the involved side is usually the first, and most constant symptom of non-masculinizing tumors of the adrenal cortex. One of the cases reported by Cahill and associates (27) showed metastases similar to those described here. However, there was also an invasion of the pericardium and endocardium, and the symptoms at the outset were referable to the heart rather than to the abdomen. Signs and laboratory findings compatible with adrenal insufficiency seem to have been borne out by the findings at necropsy. As far back as 1909, Winkler (32) recorded 2 cases of fully developed Addison's disease associated with cortical tumors of the adrenal gland. The absence of pigmentation in the present instance suggests that the medulla did not suffer in the same degree as the cortex of the gland. The hypoglycemia calls to mind the case of Anderson (33), in which the blood sugar dropped to 0.040% before ending fatally in hypoglycemic coma despite the use of glucose and epinephrin. In his case, as in this one, the tumor involved the left adrenal gland; the right "was smaller and more fibrous than usual."

Case 5. A. T., a 44-year-old male, first seen on 4/26/37, began to complain 6 months previously of nocturia, left loin pain, "severe" loss of weight and an abdominal mass. For one month he had had nausea, and sometimes vomiting, after meals. His past history was essentially negative; there was no history of carcinoma in the family.

Physical examination revealed an undernourished male with blood pressure of 120/78. All other positive findings were abdominal. A firm, smooth liver edge was palpable one finger below the right costal margin. Extending for 4 fingers below the left rib border, there was a firm, smooth, slightly movable mass. Laboratory studies (table 1) revealed a B.M.R. of +1; a sedimentation speed for erythrocytes of 9 in 15 minutes and 90 in 1 hour; an icteric index of 5; negative blood Wassermann and van den Bergh reactions; repeatedly normal results of urinalyses; a red cell count of 4.47 millions; a white cell count of 8400 with a normal differential count. The blood plasma proteins were 6.3%. Other blood chemical constituents in mg.% were: N.P.N. 24.0; creatinine, 1.3; sugar 103. Visualization of the pelvis and calices of the left kidney could not be accomplished by retrograde pyelography. The right kidney pelvis began to fill in 3 minutes and the left, 15 minutes after intravenous injection of radiopaque material.

At operation on 5/2/37, a large bluish adrenal tumor was found, which could not be removed. Material taken for biopsy was reported as "adrenal carcinoma." The patient was discharged on 5/16/37 at his own request, and attempts to follow his subsequent course have failed.

Except for age, this case corresponds well with that described by Burke (25), in which an "enlarging abdomen" was associated with similar symptoms and in which autopsy showed a fairly well encapsulated mass with hepatic metastases. The absence of clinically detectable hormonal inbalance places this case in Geschickter's (34) "asymptomatic" group of adreno-cortical tumors.

Case 6. E. W.,[1] a 37-year-old white female, first seen on 4/7/38, had not been well since the death of her husband 7 years previously, and had been under constant medical surveillance for the preceding year. In October, 1937, an attack of diarrhea was followed by symptoms which have gradually increased in severity; lassitude; easy fatigability; anorexia; insomnia; night sweats; fever of low remittent type; physical and mental irritability; precordial fulness and pain and loss of weight (approximately 25 lb. in 4 months). In February, 1938, she developed transient pain along the course of the right musculospiral nerve. In early March some substernal pain, and a dry, hacking cough appeared and have persisted.

Her past history included tonsillectomy and adenoidectomy at the age of 15; cholecystec-

[1] Seen through courtesy of Dr. Louis R. Kaufman.

tomy and appendectomy at 25, and fistula in ano at 34. Until March, 1937, her menses, established at 13, had recurred every 28 days with a moderate, painless flow, lasting 5 to 6 days, and containing no clots. Since that time her periods have been extremely irregular, usually delayed, and last but 1 to 2 days, with scanty clotted discharge, crampy lower abdominal pain and marked mental depression.

Physical examination revealed an emaciated individual of small frame, about 5 ft., 3 in. tall, and weighing approximately 95 lb. Her temperature was 99.8° F. by mouth; the pulse 86 and respirations 20. She appeared moderately dehydrated with sallow, dry skin. Other positive findings included a few scattered rather coarse râles in the upper left chest posteriorly, a small vertically placed heart, blood pressure 94/60, and a mass in the right kidney region which moved with respiration and gave the impression of being above the kidney, forcing it down.

Laboratory data collected during her stay in the hospital (table 1) showed the urine to be normal on repeated examinations. There was a moderate secondary anemia with leucocytosis. The count on admission is representative: erythrocytes 3.37 millions; hemoglobin 63%; packed red cell volume, 31%; leucocytes, 21,000, with neutrophilic polymorphonuclear cells 71% (9% immature); lymphocytes, 23%; monocytes, 4% and eosinophils, 2%. The sedimentation rate was 21 mm. in 15 minutes and 32 mm. in one hour. Results of blood cultures were repeatedly negative. The B.M.R. was +2%. Blood volume was 4450 cc.; CO_2 combining power, 42 volumes %; protein, 7.3%. The icteric index was 5. The stools were negative for occult blood, excessive fat, typhoid and paratyphoid organisms, and for intestinal parasites or their ova. Skin test results for various strains of brucella and streptococcic organisms proved to be negative. There was no response to the Mantoux reaction. No agglutination was seen with patient's blood against typhoid, parathyphoid, typhus and brucella organisms. The glucose tolerance curve was normal. Other blood chemical constituents in mg.% were: urea nitrogen, 15.1; creatinine 1.6; sugar, 153; cholesterol, 159; sodium, 341; potassium, 17.2; chlorides, 412; calcium, 10.9.

Retrograde pyelograms showed slightly forward rotation of the right pelvis and a bilateral ptosis of the kidneys, more marked on the right with narrowing and angulation of both uretero-pelvic junctions. No frank abnormality of the heart or lungs was seen in repeated x-ray examinations.

Her clinical course continued to be that of a low grade sepsis, in which respiratory distress, and the vague signs of a right sided abdominal mass apparently attached to the liver were the only localizing phenomena. The usually chronic systemic infections had been ruled out, so laparatomy was performed on 4/19/38 with the hope of finding an intra-abdominal lesion amenable to surgery, such as a perinephric abscess, a pancreatic cyst, amoebic abscess of the liver. The possibility of abdominal Hodgkin's disease, lymphosarcoma, an intestinal carcinoma (perhaps appendiceal) with hepatic metastases, or of an adrenal new growth were borne in mind. On abdominal exploration through a right paramedian incision, enlargement of the liver, which was not explained, and bilaterally atrophic ovaries, were the only positive findings Following operation bronchopneumonia developed with a preagonal temperature of 105° F., high pulse, increasing asthenia and cachexia. Sudden death, apparently of respiratory origin, occurred on 5/4/38.

Of note in a complete autopsy of the body were the primary tumor of the right adrenal and the normal size of the left; the metastases to the mediastinum and lungs; the absence, of metastases to the liver, and tracheo-bronchial lymph nodes; the small uterus and the small, atrophic ovaries.

The right adrenal was almost completely replaced by a firm, nodular, yellowish spheroid tumor weighing 350 gm., and measuring $8 \times 8.5 \times 5.5$ cm. Its thin, transparent capsule was traversed by numerous congested blood vessels. The superior surface was embedded in a concavity of the cystic lobe of the liver. The inferior surface was applied to but did not invade, the capsule of the kidney. Mesially the tumor partially compressed the inferior vena cava, and on vessel section was seen to invade the adrenal vein.

On cut section, the tumor tissue bulged slightly, exuded a thick viscid cloudy fluid, and presented a compact pale yellowish white structure containing numerous areas of a

pale light reddish brown color, and others of a spongelike consistency, particularly near the center. In the upper pole there was a small depressed wedge-shaped segment which was interpreted as a remnant of the adrenal gland.

Microscopic sections of the tumor in the right adrenal showed variously sized nests of large epithelial cells separated by small amounts of loosely arranged fibrillar connective tissue. At the periphery these were well stained while in the center they were completely destroyed. Some of them presented a small centrally placed space containing coagulated cellular debris, the so-called pseudo-alveolae, which bore a striking resemblance to the structures of the glomerulosa layer of the adrenal cortex. The tumor cell was large, had a vacuolated eosinophilic cytoplasm and a distinct cellular membrane. The nucleus was large, hyperchromatic, and in many instances, presented a single large nucleolus. A moderate number of multinucleated cells were seen. There were 4 to 6 mitotic figures per high power field. Sections of the tumor stained by the Masson technic showed no evidence of fuchsinophilia. The left adrenal gland weighed 13 gm., and, on multiple transection, the cortical layer varied from 1.5 to 2 mm. in thickness. The medulla showed a single encapsulated neoplastic nodule 2 mm. in diameter. Microscopic examination revealed a normal glandular texture.

The kidneys were normal except for the presence of numerous tumor nodules varying from pin-point to 0.8 cm. in size, which resembled the mass seen in the right adrenal.

The liver weighed 2085 gm., was markedly congested, and showed pressure, necrosis, and generalized toxic hepatosis about the concavity in which the adrenal tumor was embedded.

The uterus measured 5.5 × 4 × 2.7 cm. The left ovary measured 2.7 × 1 5 × 1 cm., the right 2.5 × 1.2 × 0.7 cm. The tunicea albugineae were markedly thickened and the surfaces nodular and fissured. The stromae were firm, pale brown in color and contained many small fibrous bodies.

On removing the anterior chest plate, a lobulated nodular, somewhat pearshaped tumor mass, measuring 8 × 7 × 3.5 cm., occupied the thymic region. The tumor with its apex directed upward, overlaid the aorta and its branches, the superior vena cava and the left innominate vein. The posterior surface penetrated the parietal pericardium, thereby coming in contact with the ascending arch of the aorta and a small section of the pulmonary artery. A bean-shaped nodulated mass of neoplastic tissue 2 × 0.7 × 0.2 cm. was found in the lumen of the left innominate vein. This was directly connected with the thymic mass via cord-like projections along the smaller thymic veins. At the superior edge of the tumor some remnants of thymic tissue were discernible. None of the lymph nodes surrounding the trachea or bronchi were involved. Microscopic sections of the growth revealed a structure similar to that found in the right adrenal.

Areas of consolidation were seen in the cut sections of both lungs. Multiple neoplastic nodules varying from 2 to 4 mm. in diameter and microscopically like those already described, were observed throughout both lungs and in the visceral pleurae.

The anatomic diagnoses included primary carcinoma of the right adrenal gland with metastases to the thymus gland and surrounding structures, the lungs, the left adrenal gland and the kidneys; advanced atrophy and fibrosis of the ovaries; chronic hepatosis; terminal confluent broncho-pneumonia.

Low grade sepsis with localizing signs and symptoms in the right loin and mediastinum were the outstanding features of this case. *Case 5* described by Cahill and associates (27) closely resembled the present instance. Their patient was a male, 57 years old, in whom progressive weakness, loss of weight, dyspnea and an abdominal mass with pain were the characteristic features. There was, however, no abdominal distress at any time in the present instance. A more rapidly fatal condition (3 months) of like kind involving the left rather than the right adrenal was described by Shenstone and Longheed (35). At postmortem examination, the findings were practically identical save that in the latter case, metastases were found in the liver. Graef's patient (15) experienced most of the phenomena here described in addition to exhibiting the signs and symptoms of Cushing's disease. Necropsy revealed

a tumor mass embedded in the cystic lobe of the liver, but distinctly encapsulated and producing a pressure atrophy similar to that already noted in our patient.

In the pathogenesis of cryptic fevers, it seems pertinent to suggest that the clinician would do well to consider the adrenal gland before eliminating all diagnostic possibilities.

Hormonal Studies

Quantitative tests for hormones have been made in 11 patients with a proven adrenal lesion, in 4 patients with secondary sexual alterations in whom no glandular disturbance has been found, and in 3 individuals with a known endocrine disturbance in which the adrenal played no recognizable rôle. The gonadotropic hormone of the anterior pituitary has been detected by the method of Zondek as described by Wolfe (36). Estimations of estrin have been carried out as suggested by Frank (12). The bitterling tests have been performed by Dr. Israel Kleiner (to whom appreciation is herewith expressed) according to a technic previously described by him and his associates (37).

All determinations of the excretion of the gonadotropic factor of the hypophysis fell within or below normal limits. Without a single exception estrin levels were high in carcinomas of the adrenal gland associated with masculinization, but in the other conditions showed no striking deviation from normal. Excessive growth of the ovipositor of the female bitterling was seen only in the 5 cases of hyperplasia. In one of these the increased response was very slight, but in the remaining 4 was unmistakable. Determinations made both before and after unilateral adrenalectomy in a female with virilism showed a reduction of the previously high values in conjunction with recession of the masculine characteristics.

It is believed that carcinomas of the cortex of the adrenal gland arise de novo or from pre-existing adenomas. That the large majority of adenomata are asymptomatic is attested by their presence in approximately 35% of all bodies coming to autopsy (38). This fact is in sharp contrast with Young's (39) ability to collect from the literature no more than 33 adults and 31 children with primary masculinizing cancer of the adrenal cortex. A year later Feinblatt (40) described 20 cases of adenocarcinoma seen personally, not one of which "presented the typical 'suprarenocortical syndrome'." Geschickter (34) found 72 instances of new growth of the adrenal cortex among the records of The Johns Hopkins Hospital. Sixty-three of these were asymptomatic, benign, small adenomata. Three other adenomata and 3 carcinomata were associated with masculinizing features. The 3 remaining tumors, all carcinomata, were clinically characterized by abdominal symptoms and malignant cachexia. If we limit ourselves strictly to carcinomatous lesions, it would seem that not more than 100 have been thus far described in the literature; of these, approximately half show no signs of Cushing's syndrome or of the adrenogenital transformation. Of 37 verified cases of "pseudo-sexual precocity" in pre-adolescent girls, Reilly, Lisser and Hinman (41) found carcinoma present in approximately 13. While Vines (26) comments that "carcinoma of the adrenal cortex is a relatively common tumor," he and his associates in 1938 report experience with but 10 proven cases. Geschickter (42) looks upon tumors of the carcinoma of the adrenal cortex as an uncommon tumor, and believes both clinical and histogenetic phases need further clarification. Haymaker and Anderson (19) have attempted the former on historical and functional grounds.

It would seem that our increasing knowledge of hormonal activities of the adrenal cortex (18–20, 26, 43–46) together with more or less specific staining reagents (26, 30, 31) will eventually lead to a more satisfactory classification. Extant evidence would suggest that the adrenal cortex secretes a "vital factor" (19, 20), a sodium regu-

lating factor (43), male hormone (27, 47, 48), female hormone (12, 26, 49–52), and other androgens found only in pathological states (27, 31).

If one should admit that these substances alone are responsible for the production of the clinical pictures seen, and that each as compared with the others, might be present in usual, diminished or increased amounts, then some 35,939 possible combinations emerge. These are far from all of the possibilities; age, sex, activity of other glands, and so forth must be considered. Moreover, despite the work of Grollman (53) and others we may yet find a separate adrenal factor regulating sugar metabolism or even blood pressure in much the same fashion that Hartman (43) has recently

TABLE 2. APPROXIMATE TITER OF HORMONES IN THE URINE IN VARIOUS PATHOLOGICAL STATES

		Diagnosis	A.P. gonadotropic hormone (R.U. per liter)	Estrin I.U. per liter	Bitterling test (ovipositor units)	Sex	Number of determinations
Normal values by method used			<25	<300	<50		
Adrenal Lesions	Carcinoma	With masculinzation	0 <10	1500 750	17 <15	F F	2 2
		With Cushing's syndrome	0	1000	25	F	1
		Without masculinization	— —	— —	25 <20	F F	1 1
		Adenoma with masculinization	15	40	<15	F	1
	Hyperplasia	With intersexuality	1 20[1] —	25 45[1] —	75 <25[1] >100	F F F	3 1 1
		With secondary sexual changes	0 0 0	32 60 50	120 90 55	F F F	1 3 1
Changes in secondary sex characteristics. No change in adrenal demonstrated			18 25 — —	35 — — 20	25 20 <25 <50	F F F F	1 1 2 1
Miscellaneous glandular disturbance without apparent adrenal or sex change			— 15 —	— — —	<30 0 <13	M M F	1 1 1

[1] Six months following removal of one adrenal from case immediately above.

isolated the sodium regulating hormone. In actuality, the clinical syndromes which have been described are relatively few in number. Some workers have attempted to group them all under the adrenogenital and pituitary basophilic syndromes. This is apparently too simple an arrangement to be feasible. For instance in the 6 cases here described, we have represented instances of Cushing's disease (*case 1*), of masculinization with superimposed Addison's disease and malignant cachexia (*case 3*), of the adrenogenital syndrome with Addisonianism (*case 2*), of abdominal tumor with malignant cachexia, but no signs of the adrenal cortex insufficiency (*case 6*), of abdominal tumor with adrenal insufficiency (*case 4*) and of simple abdominal tumor (*case 5*).

The most important feature would seem to be the establishment of criteria by

which benign and malignant lesions may be differentiated from each other. Hormone studies seem to offer the best approach to this subject. Frank (12, 51, 52) has found excessive amounts of estrin in cases of carcinoma of the adrenal cortex with and without masculinization and in no other condition of that portion of the gland. Patterson and Greenwood (26) distinguish "free" and "bound" male hormones and note a marked increase in the former in all conditions associated with "virilism" of the adrenal cortex. The bitterling test has been proposed as an estimate of androgenic substances in the urine (37). Table 2 lists the conditions in which we have had opportunity to see it tried in conjunction with estimations for estrin and prolan A and B. Whether or not the response of the female bitterling is to androgenic substances only is not known (54). A reference to table 2 would imply, however, that it is most strongly positive when hyperplasia of the adrenal is accompanied by sexual alterations. Masculinizing carcinomata seem to yield normal values. However, the group of cases so far observed is too small for the formulation of hard and fast conclusions, particularly as cases of carcinoma have been reported without an increase in male hormone (55, 56) and others with an increase (2, 27, 57–59).

In hyperplasia of the adrenal cortex, an increase in androgenic substances seems to have been the rule. Patterson and Greenwood (26) found an "appreciable amount" to "very large amounts" of "free" hormone in 11 of 14 cases of virilism without tumor. This "free" hormone is present in negligible quantities in the normal female and only up to 5 U in the male.

Increase in estrin has been looked upon as a diagnostic feature of carcinoma of the adrenal cortex, but cases have been reported in which values are normal (55, 56). In view of these facts, and until further tests are made, it is unwarranted to draw sweeping conclusions, from the increased bitterling response seen in cases of adrenal cortical hyperplasia as compared with "normal values" obtained in other lesions of the gland. It may be that the ovipositor of this fish responds to "free" and not to "bound" male hormone, although in that case one would expect to find as great a response in carcinoma and adenoma as in hyperplasia. Some as yet unidentified androgen, similar to that observed by Butler and Marrian (44) in every type of virilism (26) may prove to be present only in those hyperplasias associated with altered secondary sex characteristics.

As a matter of fact Gersh and Grollman (60) found the normal adrenal cortex totally devoid of androgenic activity and attributed the effects observed pathologically "either to the inclusion of embryonic testicular tissue or to metabolic dysfunction in which some part of the cortex elaborates an androgenic sterol chemically related to the cortical hormone." However, the presence of such tissue has not been demonstrated histologically in or about the adrenal bodies.

Perhaps it would be better to associate the secretory disturbance of the adrenal in cases with masculinization with alterations in the development and disappearance of the "x-zone." Broster and his associates (26) have shown that in the human fetus this "neutral sex zone" with peculiar staining characteristics, lasts longer in the male than in the female, and that its various phases are associated with continuously changing activity in the newly forming pituitary gland. Van Dyke (61) quotes the work of Deanesly, who suggests that the development of the "x-zone" probably depends upon a secretion of the anterior pituitary gland. This central cortical fringe appears in immature or young female mice, but fails to develop in male mice unless castration is performed. Conversely, Starkey and Schmidt (62) have been able to cause rapid disappearance of the fuchsinophilic cells by the use of testosterone propionate. Deanesly believes that the development of this zone, in the last analysis de-

pends upon a secretion of the anterior pituitary. Her conclusion is based upon the fact that the usual effects of castration in increasing the size and duration of the zone were absent in mice with hereditary dysfunction and partial pituitary deficiency. (63). Therefore it seems likely that the masculinizing features observed in cases of adrenal hyperplasia are not at all dependent upon male hormone activity but upon steroids of closely related constitution with apparently opposite or at least, different effects. It is for the biologic activity of such steroids that the bitterling test may prove to be valuable.

SUMMARY

Six cases of carcinoma of the cortex of the adrenal gland are described, in 4 of whom the diagnosis was confirmed by autopsy, and in 2 by operation and biopsy.

In 3 instances, all females, the adrenal neoplasm was associated with masculinization. Two individuals, 29 and 38 years old respectively, presented the adrenogenital syndrome, and late in the course of their disease, evidenced chronic adrenal insufficiency. The clinical picture of the third, age 34, resembled that of Cushing's "basophilism."

An abdominal mass was the only common feature of the individuals without virilism, of whom two were females, aged 70 and 37, respectively. One of the females developed Addison's disease prior to death; the other maintained a state of low grade sepsis for at least 5 months premortem. The abdominal mass and the cachexia were the only features of the disease in the male.

Estimations of estrin, gonadotropic factor of the pituitary, and "androgenic or androgenic-like materials" in the urine of 18 patients are recorded. High estrin values have been obtained only in carcinoma of the adrenal gland; increased bitterling responses in hyperplasia of the adrenal cortex.

The relative frequency of adenomata and carcinomata of the cortex of the adrenal gland is discussed, and the large number of possible syndromes which could be clinically associated with the latter, is suggested.

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THE AGE DISTRIBUTION AND SEX INCIDENCE OF HYPERTHYROIDISM AND HYPERTENSION. INTERRELATION OF THESE TWO CONDITIONS

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THIS PAPER is a study of 157 patients, 95% white and 5% Negro, which were put into two clinical groups, hypertension, 61 cases, and hyperthyroidism, 96 cases. The purpose was to shed further light on the supposed interrelation of these two conditions.

The blood pressure and the B.M.R. were determined on every patient, and as near the same time as possible. This, however, could not be accomplished in every instance and in some cases there was an interval of days or weeks between the blood pressure and the B.M.R. determinations. This introduced an error, however, repeated checks would seem to indicate that this error was not a very large one except in a few instances. It should be remembered that both the B.M.R. and the blood pressure fluctuate widely and, as Petersen has shown, the weather is an important factor. Results obtained in such a study give only approximations and should not be too rigidly applied. But in spite of all these limitations, this study does bring out some points quite definitely which we believe to be well worth recording.

The criteria used for the selection of the hypertensive cases was a persistent B.P. of 160 mm. systolic or 100 mm. diastolic or over, in patients who presented themselves for treatment. In order to make sure that we were not dealing with transitory elevation in B.P., the readings were checked 3 to 8 times at each sitting. No cases were included in the series unless they were observed for at least 3 months, during which time the B.P. did not fall below the above mentioned figures. The usual clinical criteria for the diagnosis of hyperthyroidism were followed. The technic of handling these patients is not included in this paper because it has been discussed in considerable detail in other papers (1, 2).

In the hyperthyroid group 69% had hypertension. In the hypertension group all, of course, had persistent elevated B.P., 66% had a B.M.R. above one and 23% had a B.M.R. of plus 15 or above.

Age Distribution

The distribution as to age in both groups is shown in the table at the top of the next page.

Not a single case was recorded in the 1st decade of life in either group, only 4 in the 2nd decade and 8 cases in the 3rd decade or a total of 12 cases only, in both groups, under the age of 30. In the hyperthyroid group, among the females, the greatest frequency was in the 4th, 5th and 6th decades, with 38.2% in the 5th decade alone. For the males the 5th, 6th and 7th decades had the highest incidence, with the largest number in the 6th decade.

In the hypertension group, for the females, the 4th, 5th and 6th decades were

TABLE 1

Decade	Hyperthyroid group % of all females	Hyperthyroid group % of all males	Hypertension group % of all females	Hypertension group % of all males
1st	0	0	0	0
2nd	(1 case)	(1 case)	4.3	0
3rd	7.4	8	2.1	0
4th	17.6	8	17	15.4
5th	38.2	28	21.3	30.8
6th	22.1	40	34	23
7th	14.8	12	14.9	23
8th	0	0	6.4	(1 case)

again most favored, with the largest number in the 6th decade. There were not enough males for a good comparison.

In both groups, for the females, there was a sharp increase in the number of cases from the 3rd to the 4th decade, for the males this came one decade later. There was also a similar drop off after the 6th decade. In the hyperthyroid group 60.4% fell in the 5th and 6th decades, whereas only 13 cases or 13.6% were over this age. In the hypertension group, 54% of all the cases were found in the 5th and 6th decades and only about 23% in the 7th and 8th decades. This would speak against the idea that hypertension is primarily a degenerative disease of old age.

Sex Incidence

The females far outnumbered the males in both groups. In the hyperthyroid group the females outnumbered the males about 2.75 to 1 and in the hypertension group 3.5 to 1. The preponderance of females over males may be partly due to an artifact. Women take better care of their health and have more time to visit clinics or to go to the doctor than men do. Also the author's experience has been more with adult female patients than with males or children.

In the hyperthyroid group the average B.M.R. was +31.3. For the females it

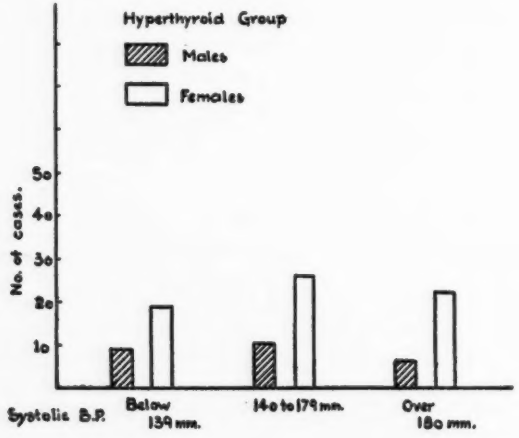

Fig. 1. GRAPH SHOWING GREATER PREPONDERANCE OF FEMALES AS SYSTOLIC BLOOD PRESSURE RISES.

was +31.1 and for the males +32.1. There did not seem to be any particular relation between the B.M.R. and the age (vide infra).

It is also noteworthy that the females greatly outnumbered the males in the brackets with very high systolic also diastolic B.P., but when the B.P. was lower this preponderance was less, or the males actually outnumbered the females. This was

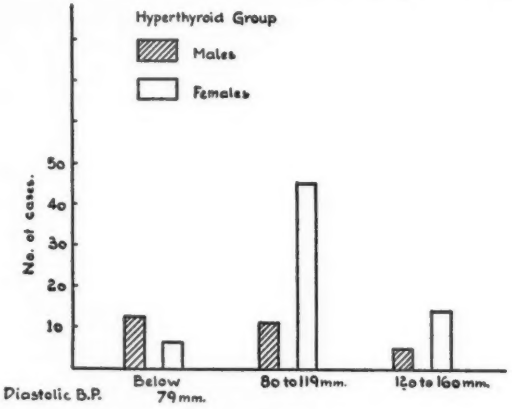

Fig. 2. Graph showing greater preponderance of females as diastolic blood pressure rises.

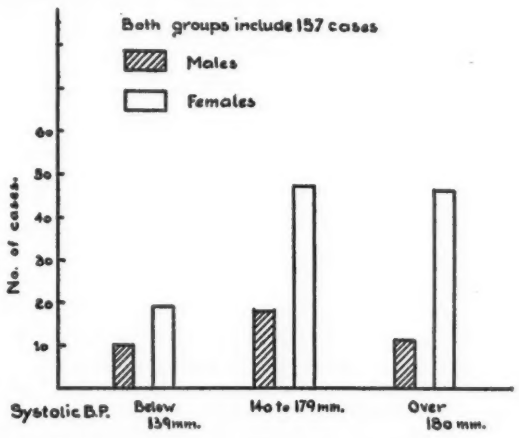

Fig. 3. Greater preponderance of females as systolic blood pressure rises.

true with one exception in both the hyperthyroid and hypertension groups. For instance, in the hyperthyroid group where the females outnumbered the males 2.75 to 1 for the whole group, the males actually outnumbered the females 2 to 1 in that group with a diastolic B.P. below 79 mm. However, when the diastolic B.P. was above 110 mm., the females outnumbered the males over 3 to 1. Above 159 mm. systolic, the females outnumbered the males about 4.5 to 1, below 159 mm. the females outnumbered the males less than 2 to 1 (see fig. 1, 2, 3).

In the hypertension group where the ratio of females to males was 3.5 to 1 for the whole group, the females outnumbered the males 7 to 1 when the systolic B.P. was above 200; 5 to 1 when the systolic B.P. was above 180, but only 2.9 to 1 when the systolic B.P. was below 180. The one exception to this was in the group below 159 mm. systolic, here the ratio of females to males was 6 to 1.

This shows that the diseases hyperthyroidism and hypertension tend to occur with a higher B.P. in the females than in the males and for this reason are more serious in females. Although very little difference could be demonstrated between the sexes in the degree of B.M.R. elevation, the differences in the vascular phenomena as evidenced by the B.P. are striking. This lends proof to the idea that the hypertension is not conditioned by the degree of thyroid disturbance but by other factors.

The Relation of the Basal Metabolism to the Blood Pressure

The following table shows the relation of the systolic and diastolic blood pressure to the B.M.R. and to age.

TABLE 2. HYPERTHYROID GROUP

Systolic B.P.	Average B.M.R. males	Average B.M.R. females	Average age males	Average age females	Diastolic B.P.	Average B.M.R. males	Average B.M.R. females	Average age males	Average age females
100–119	+35	+25	46	36	Below 70	+57	+47	40.3	47.3
120–139	+30.4	+20.9	49	44	70– 79	+35.1	+25.8	47.8	37
140–159	+32.3	+31.3	43.3	36.5	80– 89	+23	+29	53	45.9
160–179	+56	+27.5	40.5	50.6	90– 99	+32.7	+27.3	45	50.5
180–199	+31.5	+36	59.5	49.3	100–109	+16.5	+17.5	31.3	48.5
200–219	+11	+38.5	64	51.2	110–119	+24.2	+31	57.7	47.4
220–239	+24	+45.5	54.3	56	120–129	+34.5	+42.1	53.5	50.7
240 and over		+27.2		45.6	130–139		+27.5		43
					140–149		+27.8		50.5
					150–159	+31		54.5	

If we exclude the figure +56 in the second column of table 2, which is an average of 2 cases only, we observe that for the males there is a slight fall in the B.M.R. as the systolic blood pressure rises from 100 to 220 mm. For the B.P. above this point there is a rise. For the females the B.M.R. tends to rise with the systolic blood pressure from 100 to 240 mm.; but for the very high blood pressures there is a fall.

As for the relation between the age and the systolic or the diastolic B.P., both the males and the females show only a very rough tendency for the blood pressure to rise with age (table 2). Although it is generally accepted that the B.P. rises with age, we should not be too quick to decide that this holds true for hypertension. In the hyperthyroid group, the average age for the patients with hypertension was higher than those with normal blood pressure, but once we get beyond 160 mm. of Hg, the age does not rise with the B.P. in any very definite manner. Indeed when we come to the diastolic B.P. in the hypertension group, we find for the females, a

TABLE 3. HYPERTENSION GROUP

Systolic B.P.	Age, females	Age, males	Diastolic B.P.	Age, females	Age, males
150	42	43	85	54.6	—
170	45.5	51	95	51.3	54.3
190	58	46	105	49.9	48.4
210	50	62.5	115	50.5	54.5
220 and over	46.5	—	120 and over	42.5	38.5

TABLE 4. HYPERTHYROID GROUP

B.M.R.	Age	Females Systolic B.P.	Diastolic B.P.	Age	Males Systolic B.P.	Diastolic B.P.
0 to + 9	43.4	163	93			
+10 to +19	42	177	96	50.2	165	95
+20 to +29	46.3	163	93	44.2	161	101
+30 to +39	44	172	104	50.4	175	118
+40 to +49	51.4	182	104	47	137	71
+50 to +59	44.8	152	92	54.3	133	83
Above +60						

fall in the age as the diastolic B.P. rises (table 3). There was no parallel between the B.M.R. and the diastolic B.P. (table 2).

In those cases with a systolic B.P. above 220 mm. of Hg, the average B.M.R. was below that for the rest of the group. For the females it was +12.3, and for the males +24. About one-third of the females in the very high blood pressure group had a B.M.R. below one. This as well as other evidence points to an antagonism between the blood pressure and the basal metabolic rate. When the B.P. is very high the B.M.R. is apt to be low.

The B.M.R. vs. the Blood Pressure

When the hyperthyroid group was arranged according to the elevation in the B.M.R., no definite trend in age could be made out as the B.M.R. rose.

For the females there was a rise in the average systolic B.P. from 163 to 182 mm. between the B.M.R. range +20 to +49 only, above this point as the B.M.R. rose the systolic B.P. fell. For the males there was a rise from 165 to 175 mm. of Hg, for a rise in the B.M.R. from +10 to +40, but above this, the systolic B.P. fell to an average of 135 mm. This again brings out the antagonism previously referred to; the B.P. is low when the metabolism is very high (table 4).

Contrary to observations of the hyperthyroid group as regards the blood pres-

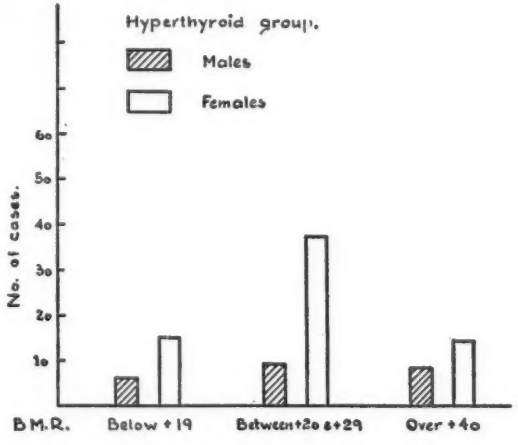

Fig 4. GRAPH SHOWING LOWER FEMALE PREPONDERANCE IN THE VERY HIGH B.M.R. BRACKETS. Female preponderance for whole group 2.75 to 1.

sure, namely that there was heavy female preponderance as the B.P. became very high; the females did not overweigh as the B.M.R. became very high. In the B.M.R. range 0 to +19 the ratio of females to males was 2.5 to 1; between +20 and +40 it was about 4 to 1 and above +40 it was only 3 to 2 (fig. 4).

The Diastolic Pressure

It is widely believed that the diastolic blood pressure in hyperthyroidism is not elevated or low. This was not substantiated by this series or by a previous series (3). In this series 58.7% of the females had a diastolic blood pressure above 90 mm. and 40% had a diastolic B.P. above 100 mm. For the males these same figures were 46 and 41.6%. The average diastolic blood pressure for the females in the hyperthyroid group was 97.4 mm. of Hg, for the males 92.8 mm. of Hg, the systolic B.P. was for the females 169 and for the males 155 mm. of Hg.

In the hypertension group 81.8% of the females had a diastolic B.P. above 90, and 54% had a diastolic B.P. above 100. The average diastolic B.P. for the females in this group was 116. In the hypertension group the systolic B.P. for the females was 187 and for the males 178 mm. The average diastolic B.P. for the males in the hypertension group was 104.5 mm. No reason can be offered for the higher blood pressures in the females.

Patients Under Observation and Treatment

There was a group of patients on whom repeated observations were made over a period of time. In this group there were 22 on whom the B.M.R. and the blood pressure were compared after thyroidectomy. There was a total of 12 patients in whom the drop in the B.M.R. was associated with a fall either in the systolic or diastolic B.P. or both. There were 2 cases in which a fall in the B.M.R. was not accompanied by any change in the blood pressure and 5 others where there was an actual rise in the B.P. with a fall in B.M.R. In 2 cases there was a rise in the B.M.R. with a fall in the systolic B.P. When the B.P. did fall after thyroidectomy it was not striking, 30 points was the maximum fall in the systolic B.P.

The sharpest drops in B.M.R. after thyroidectomy were not always associated with a similar sharp drop in B.P. In one case the B.M.R. fell from +80 to +14, but the systolic B.P. rose from 152 mm. to 160 and the diastolic B.P. from 70 to 90. In another instance a fall in the B.M.R. from +50 to +3 was associated with a rise in the diastolic B.P. of 30 points, the systolic remaining the same. In only three of the 22 cases could it be said that the fall in the B.M.R. paralleled the fall in the blood pressure. This was in sharp contrast to the quite regular fall in B.M.R. after thyroidectomy.

Although this group of cases is small, still it shows that the hypertension is not directly dependent on a thyroid toxemia. Despite the fact that in some cases the hyperthyroidism came first as to time, and the hypertension followed, it would be unsafe to conclude that hyperthyroidism is the cause of the hypertension.

DISCUSSION

The relation of hypertension and hyperthyroidism has attracted the attention of several investigators and quite a literature has sprung up on the subject. The high incidence of elevated systolic blood pressure in hyperthyroidism has been stressed (4–10), the incidence varies from 26.8 all the way to 100%. In those cases where figures on the diastolic blood pressure were included the same relation exists but in not quite as high an incidence.

From the other side of the picture an elevated B.M.R. has been reported in high incidence in hypertension (11–13). Several investigators have also alluded to what might be termed "a thyroid element" in hypertension. Riesman (14); Gallavardin (15); Plummer (16); and Mannaberg (17) have referred to it in various ways. Parkinson and Hoyle (8) think that thyroid toxemia is an underlying cause and speak of "thyrotoxic hypertension." Not all writers are agreed that the blood pressure is elevated in hyperthyroidism. Dameshek quotes Falta, Goodall, Dalmady, Leidner and Bigler and Chvostek who maintain that it is low.

Burger and Mobius found the iodine content of the blood increased in hypertension and concluded that hyperthyroidism is etiologic in hypertonia. They quote Kausch who, in a series of 272 cases of hyperthyroidism, found an elevated blood pressure in over 50%.

The idea, put forward by certain English and also German investigators, that the hypertension is dependent on the hyperthyroidism is difficult to prove or disprove since it involves several other as yet unsettled questions regarding thyroid disease. The exact etiology of the clinical entity, hyperthyroidism, is unknown. Parkinson and Hoyle delimit the problem somewhat, by stating that the hypertension is the result of a 'thyroid toxemia.' If this were true, then decreasing the thyroid toxemia by ablation of the thyroid gland or by other means should cure or at least reduce the hypertension. In other words the B.P. should show some tendency to fall with the B.M.R. This, however, is hardly the case; in many instances the blood pressure remains elevated when the B.M.R. has returned to normal and we have already referred to an antagonism between B.M.R. and blood pressure.

There is additional confirmation for the view that the diseases are related. It is well known that both hyperthyroidism and hypertension may have common origins. Pregnancy is commonly associated or followed by high blood pressure and also occasionally by clinical hyperthyroidism. Strong emotional disturbances may give rise to an elevated metabolism and even hyperthyroidism, and may also be responsible for elevation in the blood pressure as was shown by Fahrenkamp. The menopausal years, a period of both emotional and glandular disturbance, is the most common period in life for the development of both hyperthyroidism and hypertension as was shown in this paper. It is therefore not necessary to assume the hyperthyroidism as primary. It is probably better to assume that the B.M.R. and the B.P. are both under the control of a common neuroglandular system and an imbalance of this system may give rise to metabolic or blood pressure disturbances or both.

Let us consider the relation between the elevated metabolism and the elevated blood pressure in greater detail. There are several facts that stand out. The elevated B.P. remains in most cases after the B.M.R. has been reduced as by thyroidectomy. There is also other evidence of a similar nature. For instance, the B.M.R. can frequently be brought down quite readily by rest, sedatives or iodine, and although this is also observed for an elevated B.P., it is not nearly so frequent or so readily accomplished. Kurzrok has made a somewhat similar observation. He says, concerning the treatment of the menopause symptoms, that the first symptoms to be alleviated were flushes, sweats, irritability and sleeplessness; the last, arthritis and hypertension.

As stated above we also have some evidence that the hyperthyroidism occurs earlier in the disturbance, but evidence that the hyperthyroidism is the cause of the hypertension for this reason, is lacking. Putting all of these observations together and looking at this syndrome in a broad sense, we can say that we are dealing with a chronic disturbance which starts out with the clinical picture of restlessness, tremor, nervousness and an elevated B.M.R.; after a period of months or years as

the disease makes progress, other control centers, which are more stable and have resisted up until now, also become irritated and break down resulting in hypertension. There are other complications, but they lie beyond the present discussion.

Such a view is helpful in explaining certain things that are observed concerning this disturbance. The hyperthyroidism is usually more easily handled because it comes earlier in the course of the disease before the body economy has been too greatly disturbed. It explains the much higher mortality and the poorer results of thyroidectomy in persons who have hypertension together with hyperthyroidism.

It also explains the apparent antagonism between the degree of elevation of the B.P. and the B.M.R. It has been commented on by Bram that hyperthyroidism tends to burn itself out with a fall in the B.M.R. In such cases the highest observations on the B.M.R. would come in the early course of the disease when the blood pressure control was still intact and consequently normal or only slightly elevated B.P. readings would be observed. Later on, as overstimulation of the thyroid gland finally results in fatigue with a fall in the B.M.R., the blood pressure control would reach the point of maximum irritation and rising or high blood pressures would be noted.

Statistical studies on age and sex incidence etc., are rather scarce in both conditions, so that the conclusions of this paper will have to await further studies for confirmation or refutation. Palmer claims that for hypertension, the females outnumber the males 2 to 1 in the age groups above 45, 75% of all cases occur in this age group. Weiss and Prusmack (18) in a study of 1198 Negro patients with hypertension found an equal distribution in both sexes. In a control group of white hypertensives there was a slight preponderance of females. They quote Schwab and Schulze who found 'hypertensive heart disease' one and one-half times as common in Negro females. Weiss and Prusmack also found that hypertension manifests itself earlier in the female than in the male, over 46% of the females were under the age of 50. For the males 28% only were under the age of 50. The percentage incidence of the total number in the fourth decade was twice as high in females as in males.

Bloom (19) brings out the fact that hyperthyroidism may not be rare in childhood. In a series of 40 cases he had 29 under 11 years of age. This is unusually high. In Lerman's (20) 731 cases of hyperthyroidism about 15% only, were under 19 years of age. Hill claims that the incidence of hyperthyroidism is almost entirely limited to the active period of sex life.

Reasons for the relation between hypertension and hyperthyroidism are as follows. (*a*) Hypertension and hyperthyroidism are most common in the same age period, that is in the 4th, 5th and 6th decades, in other words the years of the climacteric. (*b*) Hyperthyroidism is associated with high blood pressure in a very high incidence. (*c*) Hypertension is associated with an elevated B.M.R. in a high incidence. (*d*) There is female preponderance in both conditions. (*e*) Both conditions are uncommon or even rare in childhood and early youth. (*f*) In both conditions the average systolic and diastolic blood pressures were markedly higher in the females than in the males. (*g*) Both conditions have similar symptomatology. (*h*) Both hypertension and hyperthyroidism are often brought on by the same emotional disturbances such as a sudden shock to the nervous system, pregnancy and the menopause. (*i*) Recently Petersen (21) has given support to this view; he has shown that the blood pressure and basal metabolism are influenced by meteorologic changes and that they rise and fall together.

For these reasons it is concluded that hyperthyroidism and hypertension are very closely related etiologically, as a matter of fact these two conditions may represent slightly different manifestations of the same underlying disturbance.

CONCLUSIONS

Hyperthyroidism and hypertension are closely related conditions. The idea put forward by certain English and German workers that the hyperthyroidism is responsible for the hypertonia, although there is some evidence to substantiate it, cannot be entirely accepted. It may be true in some cases.

A broader view is to consider the two conditions as resulting from a common underlying neuroglandular disturbance, the nature of which is not entirely clear at the present time, although we are not entirely in the dark about it. One might wonder how the same underlying disturbance can give rise to apparently different conditions. Here it should be mentioned that we may be misled by our own classifications and a heritage of medical thinking. The more cases one sees of hyperthyroidism or hypertension, the more one sees 'sameness' in them. We have adhered too firmly to the belief that an elevated B.M.R. determines a disease entity, and that elevated blood pressure determines another. Even though the cause may be the same in several persons, the expression of the disease may be quite different depending on the personality, sex, climate and a host of other factors. Maybe it is because of our failure to visualize disease as conditioned by these variable factors that we regard the different diseases as fixed entities.

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THE ENDOCRINE ASPECT OF ESSENTIAL HYPERTENSION AND DIABETES MELLITUS

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CHICAGO, ILLINOIS

THE RESEMBLANCE or simultaneous occurrence of these two syndromes has often been noted (1, 2). Causes of death and pathological findings common to both have also been reported (3–6). Jaffe (7) found the adrenals grossly enlarged in 18 of 65 cases of diabetes. Tumors of, or grossly enlarged adrenals have been reported as the most significant postmortem finding in cases clinically diagnosed as diabetes, Graves' disease, hypertension and chronic nephritis (8, 9).

One syndrome or the other has been reported in connection with acromegaly (10, 11), gigantism, basophilism (12), adrenal tumors (13, 14, 15), Graves' disease (15a), hypothyroidism (15b) and cancer of the thymus (16). The resemblance between Graves' disease and diabetes has been commented on particularly by Eason (17). The thyroid has been removed in the hope, and sometimes with the result, of improving the diabetic condition (18, 19). The diabetes resulting from pancreatectomy has been prevented or reduced in severity by precedent hypophysectomy (20, 21) or by suppressing pituitary function by amniotin injections (22). Injections of anterior lobe extracts have produced hyperglycemia, glycosuria and ketonuria (23). Young (24) produced experimental diabetes by injections of anterior lobe extracts. Mazer (25) reported the suppression of hyperglycemia and glycosuria in the diabetes of menopausal women by injections of amniotin. Other writers (26, 27) have thought the ovary had some power to inhibit the tendency toward hypertension, and Schaefer (28) reported a series of cases in which hypertension was relieved by injections of theelin.

Irradiation Therapy

The foregoing data and more of similar import convinced us that the pituitary and adrenals must play an important rôle in many cases of diabetes and hypertension. Because of the inhibitory or regulatory effect of the x-ray on endocrine function, we believed that this agent could be used to restore these structures to normal function or balance with a coincident obliteration of these syndromes.

The roentgenologists do not know exactly what effect their so-called stimulating doses have on the endocrine glands. Clinical observation suggests that they have a regulatory effect. The doses employed in this work are smaller than the so-called stimulating doses recommended in gynecology. As a matter of fact, they are smaller than those used by the dermatologists in treating acne and similar skin disorders.

The factors we have found most effective are as follows: 120 kv., 3 ma., 2 mm. Al filter, 50 cm. skin target distance, 50 r, measured in air, at the surface of each port of entry. Occasionally the time is extended to give 70 r to each area treated. The pituitary region is irradiated from both sides through a portal 10×10 cm. The adrenal areas are treated through a common portal 15×15 cm. The three areas are treated the same day.

Treatment has been repeated at 6 weekly intervals when needed. After a rest

of 6 weeks the series may be repeated. But if symptomatic relief or a marked fall in blood pressure occurs after the first or any other treatment, no further treatment is given until there is some return of symptoms or a beginning rise in blood pressure. Failure is most often due to too large doses, treatments given at too frequent intervals or too close to the menstrual period. In our experience treatment should not be given within 10 days prior to the menstrual period.

Our results in the past 5½ years are summarized in the tables.

TABLE 1. HYPERTENSION

Adequately treated[1]			268
Improved	186	60.4%	
Relapsed	29	10.8%	
Remained improved	157	58.6%	
Unimproved	82	30.6%	
Insufficient treatment			59
Couldn't be followed			27
			354

[1] Three or more treatments.

Criteria of improvement. Usually this has meant, in addition to symptomatic relief, reduction of at least 30 points in the systolic and 20 in the diastolic pressure. There are some exceptions to this as shown by *Mrs. M.* and *Mr. C.* In a small number the pressure has fallen to within normal limits.

Relief of symptoms has been much more striking than reduction in blood pressure and has occurred in some cases where the blood pressure has been affected but little. In a few cases blood pressure fell as much as 50 points with little symptomatic relief. In such cases the initial blood pressure was quite high and the fall seldom if ever brought it down to less than 200. So far as our observations go, such cases are not appreciably benefited by this treatment and they have been classed as unimproved.

The symptoms most often relieved have been headache, vertigo, precordial distress, irritability, pains in the legs, blurring of vision and menopausal symptoms. Tinnitus has been less regularly relieved.

Certain changes following treatment were easily recognized and more positively evaluated than the subjective sensations described by patients. These may be listed as follows: reduction in blood pressure, increase in renal function as measured by the phenolsulphonephthalein test, improvement of retinal lesions, reduction of the B.M.R. and improvement in the condition of the skin.

An improvement in vision, in the retinal lesions and in kidney function has been seen in a few cases when the course of the disease was probably unaffected by this therapy and the patient was rated as unimproved. This may be illustrated by the following case history.

Mrs. B., was 40 years of age and had known of her hypertension for 10 years. She complained of violent headaches and blurred vision and had had two "light strokes" during which her speech was affected. She was admitted to the hospital a few days after the occurrence of the last stroke, at which time her vision was so poor that she was unable to read the newspaper, even with glasses. Her blood pressure was 240/150. Her eye condition was reported as showing perivascular hemorrhages, cotton wool exudates, especially about the macular region and near the discs, "a typical picture of cardiovascular fundus, neuroretinitis." A nodular goiter was present. The B.M.R. was +30%. After 6 treatments she was able to read fine print without glasses. Her renal function on admission showed a return of 30% of the dye in 2½ hours. After a few treatments the dye excretion amounted to 60% in 2 hours. The blood pressure came down to left 180/115 and right 200/125. She observed that as her blood pressure rose, her vision became more impaired. Later part of the left adrenal was resected. It showed no signs of x-ray damage. She died within a few months, probably of cerebral hemorrhage. Autopsy was not permitted.

The case histories of a few patients who, we believe, experienced definite improvement are included here.

Mrs. H., the 55-year-old wife of a physician, was first seen April 16, 1934. She complained of vertigo, precordial distress, headache, shortness of breath and irritability. She had known of her hypertension for several years. She was the last of her family, the siblings having died of cardiovascular-renal disease. Her blood pressure was 230/130 left, 200/125 right. She was 5 ft. 6 in. in height and weighed 190¾ lb. The heart was regular with no murmurs, but there was some question as to enlargement. The vessels were neither tortuous nor hard. Results of blood count, blood and urine analyses were normal.

After 2 treatments her blood pressure on April 23, 1934, was 170/95. The vertigo and precordial distress were relieved and she felt comfortably relaxed, she said, and was less irritable She had 4 more treatments that year and on December 14, 1934, her blood pressure was 165/105. She had another treatment March 8, 1935, when her blood pressure was 170/105. September 3, 1935, her blood pressure was 160/100 and she was symptom-free. January 23, 1939 she wrote that she had had 4 comfortable years but that her blood pressure had risen recently to 190/115 and she had lately had some vertigo.

Mrs. E., age 60, was first seen on February 25, 1936. She had known of her hypertension for 7 years. She complained of nervousness, disabling headaches and visual upsets (glaucoma). There was a question of an enlarged heart, but the arteries were considered normal for her age. The glucose tolerance test showed the following blood sugars: 92.5, 170.4, 107.5 and 99.5 mg. A slight glycosuria occurred during the test. There were no other abnormal findings.

Blood pressure was 220/128 left. Following an x-ray treatment on March 3, 1936, the blood pressure fell to 160/95. On May 11, 1936, the blood pressure was 140/95 in the right arm and 145/95 in the left. She received x-ray treatments as follows: May 26, November 5 and December 15, 1936; February 10, June 3, 1937; February 7, February 22, April 18, May 31, September 14 and November 12, 1938. The blood pressure was within normal limits except just preceding these x-ray treatments. February 10, 1939 it had risen to 170/90 right and 165–180/105 left and she received another treatment. The blood pressure was 145/95 on April 12, 1939. She claims to be free of all symptoms which troubled her when she was first seen.

The next 2 cases show the marked effect of this treatment on the B.M.R. and the symptoms of hyperthyroidism. Unfortunately, it does not have this effect in most cases of Graves' disease without hypertension.

Mrs C. was seen January 17, 1934, at the age of 46. She complained of nervousness, irritability, palpitation and insomnia. She had lost some weight but had a good appetite. There was a fine tremor of the fingers and tongue but no goiter and no eye signs. The B.M.R. was +48%. Blood pressure was 170/90. After 3 x-ray treatments to the pituitary and adrenal regions her blood pressure was 145/90 and the B.M.R. was −6% on May 4, 1934. She has had 6 x-ray treatments since which kept her blood pressure about normal except when she developed an infection. We have noted that the blood pressure almost invariably rises in the presence of an infection.

Mrs. M., age 59, had a subtotal thyroidectomy for Graves' disease in 1928. There was a recurrence of symptoms for which a second operation was performed in 1929. A third operation was being urged on her in 1934 when she first came under observation. She presented the usual symptoms of Graves' disease. Her B.M.R. was +56%. She had considerable thyroid tissue including a mass about 2.5 cm. in diameter in the right lobe. Her heart had undergone some damage. It was enlarged and fibrillating and a systolic murmur was heard over the precordial region. She had some precordial pain radiating down the arms and was quite short of breath on exertion. Her blood pressure was 205/75. Treatment was undertaken for the hypertension by irradiation of the pituitary and adrenal areas. The thyroid was not irradiated. After 6 treatments at weekly intervals her blood pressure was 135/88. The B.M.R. had fallen to +18%. Seventeen months later the blood pressure was 165/90 and the B.M.R. was +14%. The mass of thyroid tissue seemed unchanged. The blood pressure and signs of hyperthyroidism were controlled by one x-ray treatment to the pituitary and

adrenals—not the thyroid—in 1936 and 3 more during 1937. When the symptoms returned they again yielded to irradiation, except those due to the heart, which was irreparably damaged when she was first seen.

Those cases classed as relapsed experienced symptomatic relief and a marked fall in blood pressure. This later returned to practically the pretreatment levels and was uninfluenced by further treatment, differing in this respect from those rated as improved. In many such cases there was no return of symptoms with the rise in blood pressure.

We have been surprised to note the wide difference in pressure in the two arms, sometimes as much as 30 points. The high reading may occur in one arm one week and in the other the next. The readings in both arms are taken at every visit. This difference does not occur, so far as our observations go, in persons with normal blood pressure.

Some cases were entirely unaffected by these treatments. A number had only one or two treatments and then disappeared. Others had a fair number of treatments (3 or more) but could not be followed to determine whether these had any effect. How much this treatment influences the course of the disease is a question that cannot be answered.

The majority of our patients were employees of the Illinois Central Railroad and their blood pressure status had been well determined by observation in the hospital. The others were seen in private practice and their status carefully determined before this treatment was begun. We realize, of course, that wide fluctuations may occur in blood pressure independent of any treatment. But in these cases reported as improved, no similar amount of reduction in blood pressure or relief of symptoms had occurred spontaneously or in the course of any other therapy.

Patients who have been irradiated without satisfactory results seem to become more susceptible to some of the vasodilators, particularly the nitrites. The same thing is said to be true of patients treated surgically. In a few cases patients became more susceptible to irradiation after unsuccessful surgical treatment. The following case has some interest in that respect.

Mrs. Y., age 34, was referred to us October 17, 1934. She had known of her hypertension for 10 years. She was troubled with headache, precordial distress, weakness and dysmenorrhea. The blood pressure was 260/155 right, 260/140 left. She had a very small sella and some excess hair on her body. Otherwise there were no significant physical or laboratory findings.

Up to May 11, 1936, she received 26 x-ray treatments. On July 13, 1936, her blood pressure was 200–205/125 in both arms. However, there had been practically no symptomatic relief and it was not felt that the x-ray treatments had been of any value to her.

Her condition gradually grew worse, although her blood pressure changed but little. On November 2, 1937 about one-fourth of her right adrenal and the celiac ganglion were removed. The splanchnic and adrenal nerves on that side were also severed. Following this there was a slight drop in blood pressure lasting several days but no symptomatic relief. The adrenal showed no signs of x-ray damage nor was any adenoma found.

Her condition continued to grow worse. Her headaches became more frequent and severe, retinal hemorrhages occurred, and precordial distress was more marked. On February 8, 1938, a similar operation on the left side was performed. The left adrenal was markedly hypertrophied but there was a question as to whether an adenoma was present. The blood pressure after some days dropped to 140/90. However, it remained at that level only a few days and when she was discharged from the hospital three weeks later, the blood pressure was 190/120. She had been given 12 x-ray treatments since the second operation. The last one was given on December 27, 1938. Her blood pressure has dropped to 174/106 and her symptoms are greatly improved. The retinal lesions have cleared up and she is undertaking social engagements not previously indulged in for many years.

Surgeons have attacked the problem of hypertension by various operative procedures, most of which reduce the functional output of the adrenals and through them may affect the pituitary. Furthermore, splanchnic resection, which is commonly used for the relief of hypertension, has also been employed by DeTakats and Fenn (29) for the purpose of improving carbohydrate tolerance in the diabetic.

A number of men have used this method of treatment with results similar to our own. Their experience so far reported to us is set forth in table 2.

TABLE 2. HYPERTENSION CASES OF OTHERS

	Cases	Cases improved
Martin	100	80
Baker	40	30
McGuffin	40	30
Boswell	12	11
Finch	12	9
Konantz	6	3
Parkhurst	5	3
Hadley	4	4
Miscellaneous	56	47
	275	217

A number of men (30) report treating patients in this way without any apparent effect on blood pressure or symptoms. So far as we have been able to learn, these men have either used much heavier doses than those recommended, given them too frequently or too close to the menstrual period.

An example of what happens when treatments are given too frequently is furnished by *Mrs. E. J.*, the 52-year-old wife of a physician. We first saw her March 13, 1935. She had known of her hypertension for 8 or 10 years. She complained of headaches, emotional instability, heart consciousness, frequent severe hot flashes and inability to think clearly at times. Her husband said that because of this she had difficulty managing her household. Blood pressure was 260/130. She had her first x-ray on March 13, 1935. After that her blood pressure dropped to 170/110 and it was still at that figure on March 26, 1935, when she said, and her husband confirmed her statement, that she was apparently entirely symptom-free. Because they had traveled a long way and desired further treatment, the adrenal region only was irradiated with the same factors used for the first treatment. Two days later her blood pressure was 230/110 and her symptoms had all returned and with about the same severity as when she was first seen. She has continued to have treatment at various times since, and her blood pressure has been maintained around 185/85 and her symptoms are much milder than they were, but she has never been quite so well as she was before the second treatment was administered.

Recently one of us (J.H.H.) talked to 3 roentgenologists. Two had not been able to duplicate our results. One said he had been using doses twice as heavy as we employ. The third said that his results were about the same as our own and that he used the same small doses we do.

Lately we (J.H.H. and E.E.M.) have seen a few cases of combined hypothyroidism and hypopituitarism with hypertension, in which the daily injection of 5 minims of pituitrin and 5 minims of antuitrin (not antuitrin-S) with one grain of thyroid daily by mouth restored the blood pressure to normal levels and relieved the symptoms.

Mr. L. D., a locomotive engineer, age 72, complained of dyspnea and swelling of his feet for 3 years with pain and swelling of his abdomen for one year; vertigo and spots before his eyes and headache for the past few months. Diurnal somnolence was marked. Examination showed obesity (weight 222 lb.) of the thyropituitary pattern; distant heart tones with a systolic murmur over the aortic area; liver tenderness and a partial ventral hernia; narrow

palpebral fissures; thin eyebrows and brittle nails. The blood pressure was right 186/90, left 180/80. There was a trace of albumen in the urine. Blood uric acid was 5.5 mg., cholesterol 195 mg. The B.M.R.'s had been −9.6% and +7%.

He was treated at first by rest, reduction diet and digitalis. This had no favorable effect on his complaints. Later, because of his obesity, diurnal somnolence, mental retardation, narrowing of the palpebral fissures, thin eyebrows, high uric acid and cholesterol, it was thought that he had a thyroid and pituitary deficiency. He was consequently given 5 minims of pituitrin. This was followed in the next 40 minutes by a fall in blood pressure from 184/84 to 144/60. The following day the injection of 5 minims of antuitrin had a similar effect. He was then given 5 minims each of antuitrin (not antuitrin-S) and pituitrin daily, and one grain of thyroid per day. On this regime his blood pressure dropped to 125/80, his edema and symptoms were relieved and he lost 35 lb. in weight.

We have studied the B.M.R. and the glucose tolerance of a number of hypertension patients. The results of these studies are shown in tables 3 and 4.

Table 3. Basal metabolic rate

Lower than −5%	57
−5% to plus 10%	70
Over plus 10%	58
	185

Table 4. Glucose tolerance tests

Blood sugar rose to		Glycosuria
Less than 170 mg.	75	4
170–200 mg.	46	11
Over 200 mg.	72	28
	193	43

Whether the high renal threshold indicates some renal lesions not otherwise demonstrable we do not know. The response of patients who had both hypertension and diabetes to this type of treatment is shown in table 5.

Table 5. Hypertension and diabetes

Improved as to both	13
Improved as to hypertension only	7
Improved as to diabetes only	4
Improved but relapsed	1
Unimproved	2
Insufficient treatment	6
Couldn't be followed	1
	34

By improved we mean the diabetic syndrome greatly improved or entirely disappeared and the symptoms and blood pressure were influenced as mentioned under table 1.

An example of the response of such patients to this type of therapy is given in the following case history.

Mr. C., a lawyer, age 52, was referred to us on December 21, 1936. His diabetes was discovered in May 1936, at which time he had a severe acidosis; hypertension had been present 4 or 5 years; no symptoms except that the left leg was cold and inclined to drag since a "spell" in May of 1936. Diet contained carbohydrates 90, proteins 60, fats 90, insulin 22. Blood pressure was 170/90. Blood sugar after breakfast and insulin was 169 mg. Weight was 155 lb. The general examination was otherwise not significant. The results of urine analysis, blood count and blood analysis were otherwise normal. One x-ray treatment over the pituitary and adrenal regions was given December 22, 1936. He took his last dose of insulin December 24, 1936, and added 40 gm. of carbohydrate to his diet. His blood sugar readings afterward were as follows

12/24/36	74.3 mg.	12/30/36	87.7 mg.
12/26/36	151.1 mg.	12/31/36	84.3 mg.
12/28/36	141.0 mg	1/2/37	71.1 mg.
12/29/36	119.6 mg.		
	99.0 mg.		

His blood pressure fell to 120/70 on December 24, 1936 and rose to 150–160/95–105 on January 2, 1937. He was seen again December 13, 1937 when his blood pressure was 165/95 left, 180/105 right. He was given another x-ray treatment December 13, 1937. His blood pressure December 18, 1937 was 160–145/80; blood sugar 115 mg.; weight 186 lb. February 6, 1939 his blood pressure was 145–150/84 and his blood sugar was 92.1 mg. A letter dated March 6, 1939 says his blood pressure is 166/82.

An example in which the hypertension seemed to be relieved but the diabetes was practically uninfluenced is furnished by *Mrs. P.*, who was known to have had diabetes at the age of 56. It was mild and she was able to take an adequate diet without glycosuria or hyperglycemia. Her blood pressure continued normal until July 1935, when she was 64 years of age. It is fairly certain that within a period of 6 months her blood pressure rose from about 130/90 to 220/120 and in approximately the same period her renal threshold for sugar rose to about 300 mg. The blood pressure dropped to 145/85 after a few x-ray treatments. Her blood sugar, however, responded very slowly and then only temporarily to this treatment. Her renal threshold was lower after x-ray treatment. She was compelled to begin using insulin August 1937, 10 years after her diabetes was discovered.

Results in the human diabetic without hypertension have not been as good as among hypertensive patients or in the experimental animal. Barnes (31) irradiated the pituitary and adrenal regions of a series of pancreatectomized dogs. He used the same machine and about the same factors of treatment as were used in the treatment of our patients. The severity of the diabetes was reduced on an average of 50%. Barnes was of the opinion that most of the results were due to the effect of the irradiation on the adrenals.

While most diabetics have shown no response to this therapy, a few have been definitely benefited as indicated by the following case histories.

The diabetes of *Mr. S. G.*, was discovered in 1926 when he was 42 years of age and weighed 200 pounds. He could not be persuaded to stay on any reasonable diet and October 9, 1929 his blood sugar was 200 mg. During an attack of rheumatic fever in 1931 he began taking insulin, 10 U night and morning. This continued without change and with little supervision until 1933. In December of that year he was given 2 x-ray treatments. After the second treatment he became sugar free and discontinued the use of insulin. During the Christmas holidays of 1933 his diet was even less restricted than usual and he developed mild glycosuria. A third x-ray treatment to the pituitary and adrenal regions was given January 19, 1934. He shortly became sugar free and has continued without glycosuria or hyperglycemia.

Mrs F., 62 years of age, had had diabetes for 3 years when she was first seen December 27, 1933. She had been in the hospital twice for acidosis and infections and had been unable to work for 2 years because of pain in her shoulders and arms. She was taking 20 U of insulin per day. After 4 x-ray treatments she became sugar free and had a normal blood sugar and stopped using insulin. She has had frequent blood sugar determinations since these treatments were given. In every case the sugar has been just above normal except in December 1935 when it was 180 mg. Of course it is not unusual for diabetics to experience an increase in carbohydrate tolerance and be able to discontinue using insulin. However, they rarely do so while gaining weight. This woman has gained 20 lb. since x-ray treatments were started.

About one-third of the diabetics have experienced some symptomatic improvement and an increase in their carbohydrate tolerance; another third have claimed symptomatic improvement but have shown no change in carbohydrate tolerance. The others have been entirely unaffected.

Safety of this procedure. This treatment has been criticized (32, 33) on the ground

that it is dangerous and apt to damage the pituitary or adrenals or both so that the patient would merely exchange his hypertension for pituitary deficiency or Addison's disease.

The tolerance of these and their neighboring structures to much larger doses of irradiation was determined by other men long before this work was undertaken (34, 35). Men who have made this criticism either have paid no attention to the dosages we have used or have not been familiar with x-ray dosage.

Furthermore, in our experience, heavier doses, still not within the bounds of that heavy enough to do any harm, have not been effective in relieving symptoms or reducing blood pressure. Some four years ago we gave to a few patients heavier doses using the following factors.

Part	KVM	Port	Filter	STD.	Ma.	Time	Dosage in r
R. pit.	180	10×10 cm.	1 mm. Al. 0.25 mm. Cu.	50 cm.	5	15 min.	140
L. pit.	180	10×10 cm.	Same	50 cm.	5	15 min.	140
Adrenals	180	15×15 cm.	Same	50 cm.	5	20 min.	190

These treatments were invariably followed by headache, vertigo and weakness. Tinnitus and nausea occurred occasionally. There was no relief of the symptoms associated with the hypertension and practically no reduction in blood pressure. After waiting 6 weeks, these patients were given the usual small dose already mentioned. These small doses were followed by relief of symptoms and reduction in blood pressure.

In 2 of those cases receiving heavier doses we were able to secure biopsy material from the adrenals; in one the pituitary was removed at the postmortem examination. The pathologists reported that the sections showed no sign of x-ray damage. There has never been any clinical evidence of pituitary or adrenal damage.

An indefinite number of x-ray treatments can be given with no ill effects. We have given more than 40 treatments each to a few patients without the slightest sign of damage.

It has been said that results following this therapy are due to suggestion and not to the x-ray treatment. There is no way of proving conclusively that this is not partly true. But we have tried to minimize the effect of psychotherapy; and, preceding the giving of the heavier doses which were not effective, we suggested to the patient that the doses were being changed as to be much more potent.

CONCLUSION

Essential hypertension and diabetes mellitus resemble each other in so many respects as to suggest common etiological factors in many cases. The endocrine components appear to be the pituitary, adrenals and thyroid. Low-dosage irradiation applied to the pituitary and adrenal regions has been followed by symptomatic relief and a considerable reduction in blood pressure in most cases. In some this reduction in blood pressure has been a temporary affair which could not be maintained by subsequent treatments given when indicated by a rise in blood pressure. In many cases the symptoms did not recur when the blood pressure returned to the pretreatment level. A few cases of hypertension have been seen in association with pituitary and thyroid deficiency. In these the blood pressure returned to normal levels and symptoms were relieved following the administration of thyroid and extracts of the anterior and posterior lobes. In a small series of patients having both hypertension and diabetes the latter appears to have been greatly improved in 50% of the cases, following irradiation of the pituitary and adrenal regions.

This form of therapy is an entirely safe procedure and can do no patient any harm if used in the dosages and by the methods we have employed. It seems to possess sufficient promise to warrant careful investigation by other workers.

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30 NORTH MICHIGAN AVENUE
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ESTROGEN METABOLISM IN CANCEROUS AND NON-CANCEROUS WOMEN[1]

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IT HAS BEEN SHOWN that the urinary estrogens of normal non-pregnant women, when extracted after optimum conditions of hydrolysis and separated into estrone (weak phenolic) and estriol (strong phenolic) fractions, will vary in concentration during the menstrual cycle, and almost invariably have higher activity in the estrone fraction (1, 2). In rabbits (3, 4) injected estrone becomes in part converted to estriol in normal females but not in ovariectomized females or in females lacking uteri. The simultaneous injection of estrone plus progesterone into rabbit females results in an increased urinary estrogen output (5) and a furthering of the estrone-estriol conversion (3). This appears to occur also in women (2).

We have determined the urinary estrogen in a series of 5 non-cancerous and 7 cancerous women before and after the injection of estrone (2.1 mg.) and estrone (2.1 mg.) plus progesterone (10 mg.). The hormones were administered in a single intramuscular injection. The urines were collected for 48 to 72 hours before injection and for 72 hours after injection. The Smith and Smith (6) method of hydrolysis was employed and the extract fractionated into estrone and estriol fractions after the method of Cohen and Marrian (7). Bioassays were conducted by a slightly modified Astwood technic (8).

The data on urinary estrogen are presented in table 1. They demonstrate the following. (*a*), That in the non-cancerous women the total urinary estrogen output is less than in the cancerous women, averaging 24γ estrone-estriol equivalent in 72 hours, compared with an average of 165γ in the cancerous group. (*b*), That the estrone fraction shows more activity than the estriol fraction in the non-cancerous group whereas in the cancerous group 4 of the 7 women show higher activity in the estriol fraction. (*c*), That after estrone administration the non-cancerous women uniformly excrete 200% to 500% more estrogen whereas the cancerous women show great variability in the increase, and on the average less than the non-cancerous. (*d*), That the ovariectomized, menopausal, and hysterectomized non-cancerous women after estrone administration gain chiefly in activity in the estrone fraction whereas the normally cyclic women gain in the estriol fraction more markedly. (*e*), That the cancerous women gain in both fractions after estrone administration. (*f*), That a further increase in urinary estrogen is given by the non-cancerous women upon the simultaneous administration of estrone and progesterone whereas the cancerous women show little or no gain. (g), That non-cancerous ovariectomized, menopausal, and hysterectomized women show practically no increase in the estriol fraction after the administration of estrone and progesterone.

From these data we deduce that normal pre-menopausal women appear to convert estrone to estriol and that this conversion is enhanced by progesterone. Apparently

[1] Aided by grants from the Ittleson Foundation and the Works Progress Administration (Project No. 665-14-3-726).

the lack of ovaries (or uterus) or the menopausal state prevent this conversion but do not prevent enhancement of total estrogen output due to progesterone. In cancerous women the evidence for conversion of estrone to estriol is conflicting and the lack of any great increase in estriol output after simultaneous estrone-progesterone administration is contradictory to such a possibility. The additional fact that progesterone scarcely increases total estrogen output inclines one to believe that the normal course of estrogen metabolism is upset in cancerous women. The data on the cancerous women would be more explicable if we assumed that they excrete estrogen which is neither estrone nor estriol but possibly some metabolic product of native estrogen. Estradiol is debarred since it is extracted into the estrone fraction (2). This putative urinary estrogen would be distributed between the estrone and

TABLE 1. THE URINARY ESTROGEN (ESTRONE AND ESTRIOL FRACTIONS) PER 72 HOURS IN 5 CONTROL AND 7 CANCEROUS WOMEN

Patient No.	Condition	Before estrone administration			After administration of 2.1 mg. estrone				After administration of 2.1 mg. estrone plus 10 mg. progesterone			
		estrone γ	estriol γ	total γ	estrone γ	estriol γ	total γ	increase %	estrone γ	estriol γ	total γ	increase %
1	Normal postmenstrual	16	12	28	55	47	102	264	153	182	335	228
2	Normal postmenstrual	26	4	30	57	36	93	210	102	130	232	149
3	Ovariectomized	12	3	15	60	17	77	513	178	13	191	148
4	Menopausal	19	4	23	112	8	120	422	242	6	248	107
5	Hysterectomized	22	2	24	80	6	86	260	315	9	324	265
6	Cervical carcinoma-pregnant 8 months	68	24	92	350	63	413	349	520	204	724	89
7	Post-menopausal cancer of fundus	100	181	281	721	380	1091	388	694	549	1243	13
8	Ovariectomized carcinoma of cervix	121	116	237	153	246	399	68	162	211	373	−6
9	Post-menopausal cervical carcinoma	15	132	147	51	109	160	9	31	112	143	−11
10	Hysterectomized post-menopausal adenocarcinoma of vagina and cervix	27	75	102	34	103	137	34	58	109	167	22
11	Pre-menopausal cervical carcinoma	128	78	206	113	117	230	11	126	102	228	11
12	Post-parturient cervical carcinoma	42	48	90	24	46	70	−22[1]	36	24	60	−14

[1] This patient was given deep x-ray therapy on the day that estrone was administered.

estriol fractions and, if it be a breakdown product of estrone, is either excreted in relatively limited amount after estrone administration or is less active by our test than estrone or estriol.

In the course of extracting the urines of the cancerous women we observed that a purple pigment was extracted into our phenolic fractions. This pigment was distributed between the estrone and estriol fractions much as we would expect the estrogen we hypothecated as the urinary product in the cancerous group. Accordingly we undertook the concentration and purification of this pigment and at the same time made a survey of its occurrence in a variety of urines from cancerous and non-cancerous patients.

In our extraction procedure an ethereal extract of the hydrolized urine is washed with sodium carbonate and then extracted 4 times with N/10 NaOH. The purple pigment goes slowly into the N/10 NaOH and comes out almost quantitatively upon backwashing the alkali with ether. Other urinary pigments taken out by N/10 NaOH are less easily removed by the ether backwash. Any of this purple pigment remaining in the neutral fraction is removed rapidly by 1/N NaOH in one or two washes. The pigment is rapidly decolorized by alkali so that the 1/N NaOH wash must be acidified at once. The ether backwash of the N/10 NaOH is added to an ether extract of the acidified 1/N NaOH, washed with Na_2CO_3 and H_2O and the

color of the extract determined. The purple pigment is ordinarily easily discernible but may be somewhat obscured by a red pigment also found in this fraction. We shall elsewhere go into a detailed description of the chemistry of these two pigments. A third, usually occurring in this fraction of normal urines, is yellow, probably urochrome.

In tables 2 to 4 we present in detail the data on a series of cancerous and non-cancerous women and men. These data are summarized in table 4. A urine containing the purple pigment is designated +, one without it −, one containing traces ±. In those individuals with more than one designation those succeeding the first refer

TABLE 2. THE OCCURRENCE OF A PURPLE PIGMENT IN A "WEAK" PHENOLIC FRACTION OF URINE OF A SERIES OF CANCEROUS WOMEN

Patient	Age	Condition	Urines
S.Y.	33	squamous cell epithelioma of cervix, ovariectomized	+++
D.R.	69	adenocarcinoma of fundus extended to stump of cervix after supravaginal hysterectomy	+++
R.O.	33	squamous cell epithelioma of cervix	+++
B.R.	69	carcinoma of cervix	+++
C.R.	68	carcinoma of fundus	+++
S.P.	30	carcinoma of cervix	+++
F.I.	65	adenocarcinoma of body of uterus	±++
P.E.	50	carcinoma of cervix	+++
G.O.	53	squamous cancer of cervix (Radium)	+
O.L.	34	squamous cancer of cervix removed by hysterectomy	+
G.A.	68	carcinoma of cervix	+++
R.H.		adrenal tumor	+
P.A.	60	cancer of cervix	+++
H.I.		adrenal tumor (?)	+
K.O.	66	cancer of breast	+
T.A.	49	cancer of cervix	+
D.O.	51	squamous cell carcinoma of cervix	+++
S.N.	58	adenocarcinoma of uterus	−++
B.E.	42	carcinoma of cervix after radium and x-ray	±
G.O.	72	cancer of rectum with metastases	+
M.A.	57	adenocarcinoma of uterus	+
G.R.	56	squamous cell epithelioma of endocervix	+++
D.I.	62	cancer of cervix	±++
S.E.	61	cancer of breast	+
R.O.	75	gastrointestinal malignancy	+
R.I.	74	gastrointestinal malignancy, caecostomy	±
B.L.	36	cancer of head of the pancreas	+++
R.I.	77	epidermoid cancer	+

to collections made after estrone injection and after estrone plus progesterone respectively.

It is obvious from these data that the pigment occurs preponderantly in the urines of cancerous patients. Among the cancerous women the pigment was not observed in the urine of one woman (*S.N.* table 2) recently given radium and deep x-ray therapy and did appear in this same patient's urine after estrone administration. Among the cancerous men the pigment was not found in the urine of a case of cancer of the throat (*A.L.*, table 4) and a case of cancer of the ethmoid (*C.R.*), both patients with active malignancies, nor in the urine of 3 cases declared "cured" (*L.I.*, *S.T.*, *G.E.*). In one of these 3 (*L.I.*) the pigment reappeared after hormone injection.

Of the non-cancerous women 5 patients excreted the pigment and it is notable that all of them are over 40 years of age. Of the 4 non-cancerous males excreting the pigment 2 were boys with rheumatic fever (excreting traces only) and the other 2 young men, one with peptic ulcer, the other asthmatic.

TABLE 3. THE OCCURRENCE OF A PURPLE PIGMENT IN A "WEAK" PHENOLIC FRACTION OF THE URINES OF A SERIES OF NON-CANCEROUS WOMEN

Patient	Age	Condition	Urines
C.A.	20	Ovariectomized	−−−
P.I.	38	Normal	−−−
M.I.[1]	30 (?)	Toxemia of pregnancy	−
W.E.	32	3 to 4 months pregnant	−
H.O.	42	In menopause	−
P.I.	38	Normal at menstruation	−
M.I.	29	Normal	−−−
H.E.	22	Normal	−
T.O.		Amenorrhea	−
B.E.		Dysfunctional flow	−
N.A.	26	Bleeding at coitus	−
V.I.	18	Benign tumor of breast	−−−
D.O.	92	Coronary thrombosis	−
S.U.	63	Heart trouble (?)	±
O.M.	46	Hysterectomized	−−−
F.I.	55	Cerebral hemorrhage, accident	+++
A.L.	70	Diabetes mellitus	−−
R.O.	41	Acne, estrone administered	+
D.U.	58	Asthma	−
F.A.	68	Arteriosclerotic heart disease	+
B.E.	35	Pneumonia	−
G.I.	45	Hysteria	+

[1] Received estradiol benzoate and progesterone.

DISCUSSION

It is obvious from these data that the cancerous women of our experiments metabolize the estrogens in an abnormal manner. Most striking is the slight or negligible

TABLE 4. THE OCCURRENCE OF A PURPLE PIGMENT IN A "WEAK" PHENOLIC FRACTION OF THE URINES OF A SERIES OF CANCEROUS MEN

Patient	Age	Condition	Urines
L.I.	44	Basal cell carcinoma of skin; x-rayed and no recurrences	−++
D.A.	60	Malignant melanoma of thigh with metastases	−++
S.T.	60	Basal cell carcinoma of nose; x-rayed, no recurrence	−−−
M.K.	82	Cancer of sigmoid	+++
A.L.	55	Epidermoid cancer of right foot	+++
K.A.	59	Cancer of kidney	+
G.E.	55	Embryonal cell cancer of testis; removed, no recurrence	−
K.U.	43	Sarcoma of the ileum	+
D.A.	65	Cancer of stomach	+
P.O.	78	Squamous cell epithelioma of penis	+
C.O.	56	Cancer of left kidney	+
W.A.	43	Cancer of bladder	+
H.E.	58	Cancer of bladder	+
G.I.	—	Adrenal tumor (?)	+
B.R.	44	Adenocarcinoma of descending colon	+
A.L.	60	Cancer of throat	−
W.E.	66	Cancer of stomach	+
M.E.	64	Intraabdominal malignancy	+++
D.O.	70	Carcinoma of lip	+
C.A.	52	Cancer of pharynx	+
B.L.	78	Cancer of stomach	+
C.R.	37	Carcinoma of ethnoid	−
H.A.	72	Cancer of sigmoid; colostomy	±
L.O.	43	Cancer of right lung	±
B.R.	62	Cancer of bladder	+
H.I.	72	Adenocarcinoma of sigmoid	+
L.A.	68	Adenocarcinoma of sigmoid, tumor removed	±

TABLE 5. THE OCCURRENCE OF A PURPLE PIGMENT IN A "WEAK" PHENOLIC FRACTION OF A SERIES OF NON-CANCEROUS MEN

Patient	Age	Condition	Urines
W.T.	29	Normal	−
P.N.	36	Normal	−
G.R.	37	Normal	− − −
M.A.	11	Rheumatic fever	±
C.O.	46	Coronary thrombosis	−
H.I.	45	Bleeding peptic ulcer	−
J.E.	63	Cerebral hemorrhage	−
M.C.	12	Rheumatic fever	±
P.A.	36	Asthma	+
F.J.	25	Peptic ulcer	+

increase of total estrogen output after estrone plus progesterone administration. In all 4 experiments with women having functional uteri as well as in those of Smith and Smith (2), the combined administration leads to increased total estrogen especially in the estriol fraction.

We have sought for an explanation of this in the postulation of an estrogen which is not one of the recognized native hormone products but some abnormal metabolic product apparently partitioning between the estrone and estriol fractions. The solubility behavior of the purple pigment occurring predominantly in the urines of cancerous patients is that of the postulated estrogen. But our data may be equally well explained by assuming that a rather marked destruction of injected estrone occurs so that the normal result is not obtained. In our minds this alternative explanation is not quite consistent with the rather large excretion of estrogen activity in these women before estrone injection. We are further encouraged in this idea by the fact that the pregnant woman with carcinoma of the cervix (*case* 6, table 1) excreted very low amounts of hormone for the stage of pregnancy, e.g., 92 micrograms equivalent compared to about 9000 micrograms equivalent usually obtained (1, 9). This would indicate that endogenous estrogen is converted to material of low estrogenic potency. It is notable that the pigment output of this patient was also large. While we have yet to establish methods for the exact quantitative measurement of the pigment, it can be stated that the largest quantities were observed in the earliest cases of untreated malignancies.

It cannot be claimed from our evidence that the purple pigment is the putative estrogen. Experiments now in progress indicate that the material can be obtained free of estrogen, and we have in fact obtained a small amount of needle-shaped crystalline pigment. As soon as sufficient quantities are available we shall be able to determine its chemical constitution accurately so discussion might as well be deferred until fact is available.

TABLE 6. SUMMARY OF THE DATA ON THE OCCURRENCE OF A PURRLE PIGMENT IN A "WEAK" PHENOLIC FRACTION OF THE URINES OF CANCEROUS AND NON-CANCEROUS MEN AND WOMEN

Sex	Type	No. patients	No. urines	No. positive	% positive urines	% positive patients
♀	Control	22	35	7	20	23
♀	Cancerous	28	58	57	98	100
♂	Control	10	12	4	33	40
♂	Cancerous, not cured	24	32	29	91	92
♂	Cancerous, "cured"	3	7	2	29	33
♂ and ♀	Control	32	47	11	24	28
♂ and ♀	Cancerous	52	90	86	95	96

It should be pointed out that increased serum estrogen in certain cancerous women has been reported previously (10) and in the urine of certain patients with malignant growths (11). But all of these observations were made with extracts that were imperfectly prepared by modern standards, e.g., conditions of hydrolysis necessary for maximum estrogen yield were not employed. Burrows, Cook, Roe and Warren (12) have isolated Δ 3:5 androstradiene-17—one from the urine of a man with a malignant tumor of the adrenal cortex. This compound is weakly estrogenic. It has not been reported from other urines.

SUMMARY

The urinary estrogen was determined quantitatively before and after the administration of estrone and estrone plus progesterone in 5 non-cancerous women and in 7 women with either cancer of the cervix or fundus. The 2 non-cancerous women with normal functional ovaries and uterus appear to convert injected estrone in part to estriol and this conversion is enhanced by progesterone injection. The 2 non-cancerous women with presumably non-functional uteri and the hysterectomized woman do not appear to effect this conversion but, like the normal non-cancerous women, do excrete more estrogen after estrone plus progesterone injection than after estrone alone. The cancerous women appear unable to effect the conversion of estrone to estriol to any appreciable extent even with progesterone injection and this in spite of the fact that their total estrogen output before injection is higher than the control group. It is deduced that the cancerous women may convert both endogenous and injected estrogen into a compound of low estrogenic potency.

The occurrence of a urinary pigment having the solubilities of the putative estrogen is described in a series of 28 cancerous women, 27 cancerous men, 22 non-cancerous women and 10 non-cancerous men. One hundred per cent of the cancerous women, 92% of the cancerous men, 23% of the non-cancerous women and 40% of the non-cancerous men excreted this pigment in varying quantity.

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ALOPECIA TOTALIS

WITH SPECIAL REFERENCE TO FAMILIAL AND ENDOCRINE FACTORS

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Following the use by Bengston (1) of anterior pituitary extracts with some success over a prolonged period of time on a group of 16 patients suffering from alopecia areata and totalis, Lord (2) using the same therapy, had disappointing results in all of 14 cases of advanced seborrheic alopecia. In 3 of 5 instances however, of alopecia areata similarly treated, complete regrowth occurred within periods ranging from 3 to 6 months. The remaining 2 patients who submitted only to ultraviolet irradiation and to topical applications of cresol and alcohol also experienced recovery within 7 to 9 months.

Kohn (3) was successful with anterior pituitary therapy in early common alopecia, but long standing cases gave generally unsatisfactory results. Janson (4), referring to the work of Burchardt and Kun, who were using progynon to promote hair growth in presenile and senile male rats in areas previously bald, utilized this mode of therapy with some success in sexually mature women, some of whom suffered merely from thinning of hair due to illness, and some who had a frank alopecia areata.

Hocker (5) further emphasized the close relationship existing between imbalances within the pituitary-gonadal system, as illustrated by a case of acromegaly with amenorrhea, accompanying the alopecia totalis. While large doses of progynon given parenterally and orally served to reestablish the menses as well as the scalp hair, the patient later became refractory to treatment, losing her hair as well as her normal catamenia. In contrast to this, another case of Janson's (6) of severe alopecia areata in a young girl with signs of irregular menses and dysmenorrhea, responded with a permanent regrowth of hair and normal menses when progynon-B oleosum was administered.

Versari (7) calls attention to the loss of hair over the body in myxedema, while the scalp remains normal, a contradictory observation borne out by the work of Gordon and Buschke who cured alopecia in young adults with thyroid preparations.

In the face of these interesting, and sometimes conflicting reports by different authors on the effects upon alopecia areata and totalis of endocrine therapy, the following case history is presented without attempting to place it in a definite endocrine category, nor to advance a new or specific form of therapy. A few interesting and possibly significant points in the patient's earlier history as well as familial endocrinopathies may serve to shed additional light on this subject.

CASE REPORT

Mrs. A. P., an American born white female of English and German extraction, age 32, entered the clinic in June 1937. At this time she was 3 months pregnant and had lost all the hair of the scalp, eyebrows (save for a small patch over the right eye), lashes, axillae, and pubes as well as the lanugo of the face and trunk (in the order of their disappearance) in the previous 5 months.

Family history. The patient's father and one sister had had thyroidectomies performed for pressure symptoms. Another sister, 8 years older than *Mrs A. P.*, began losing her

hair in spots over the scalp during early childhood, with spontaneous return when shaved. With her first two pregnancies, the alopecia became complete, returning shortly after delivery, and at the present time she has only a few patches of hair left on her head. Her third child is 4 years old and normally developed physically, but is unable to sit up, walk alone or talk. One brother of the patient is 6 ft. 3 in. tall, weighs 230 lb. and has very large hands and feet, and a heavy lower jaw. All other members of the family save the patient and her one sister have luxuriant growths of scalp hair. There is no consanguinity of the parents, nor any other significant endocrinopathic history.

Catamenia. The menarche began at 11 years of age and has been regular but scanty and very painful. She was unable to conceive during the first years of her marriage.

Previous illness. Thyroidectomy was performed in 1931 for relief of pressure symptoms. No previous systemic diseases or debilitating illnesses have occurred. She has been taking 4 grains of desiccated thyroid since the operation.

Present illness. In the first months of 1934 a small patch of baldness was found on the scalp which, however, rapidly filled in with no therapy and she became pregnant shortly afterward, being delivered in December 1934 of a normal male child. Though nursing the baby, she began menstruating one month later and the periods were more scanty and painful than before. In January she noted another small patch of alopecia, which had increased in size by February 1937, at which time the photograph was taken (fig. 1). The eyebrows, lashes and other hirsuties were then intact. Late in March (in which month she had again become pregnant) of that year so much hair had fallen out that the head was shaved. After her menarche she became obese over the lower abdomen and hips, and following the birth of her first child, large pads of trochanteric fat appeared which she has never lost.

Physical examination. In June 1937, the patient was a short, completely bald (fig. 2, 3), small-boned woman weighing 170 lb. with heavy depots of fat over the abdomen, iliac crests and particularly the trochanters and lower legs. The upper portion of the trunk was not correspondingly heavy (fig. 4). The teeth and nails were normal, and the skin was fine, moist and hairless. The hands and feet were very small. The eye grounds revealed normal retinal vessels and discs. The B.P. was 126/60 and the pulse 96/minute. Sella plates showed normal dorsum sella and clinoids. The blood sugar curve suggested a decreased tolerance without glycosuria, the high point being reached at the end of one hour (the fractionated method of 2 50-gram doses of glucose being given within the first hour) and returning to 81 mg.% at the end of the sixth hour: fasting, 118 mg.%; ½ hour, 190 mg.%; 1 hour, 250 mg.%; 2 hours, 175 mg.%; 6 hours, 81 mg.%. Serum calcium was 8.5 mg.%. The result of a Wassermann test was negative. Results of urine and blood analyses were normal.

Therapy. Because of the patient's pregnancy, no endocrine therapy was instituted other than the 4 grains of thyroid which she continued taking following her thyroidectomy in 1931, for fear of terminating or altering the course of her gestation. She failed to return until the baby was 4 months old (at which time it was breast fed). She was still completely bald. Her menses had begun again, being characteristically painful and scanty, though regular. In June 1938 she was placed on anterior pituitary extract (Squibb) 2 cc., 3 times a week hypodermically. Because this dose caused the breasts to engorge, possibly due to the lactogenic fraction in the product, the amount was cut to 1 cc. daily for 4 days a week. After 26 days of this therapy, no massage or stimulating lotions being used, a very fine colorless lanugo appeared on the scalp, 1/10 to ⅛ inch in length. There were also a few single, long white and occasional black filaments noted. After two months of this treatment (32 cc.) the patient stopped the extract because her breasts became too painful, and she did not return until January 9, 1939.

At this time, about 18 patches of dark firmly attached silky, symmetrically spaced areas of hair had appeared on the scalp, and there was also a return of part of the eye lashes and facial lanugo. On February 15, 1939 the patient was placed on progynon-B, Schering, 2000 rat units intramuscularly every 4 days for 6 injections, as well as progynon-DH, 50 rat units daily by mouth. The menses which had previously been scanty and dysmenorrheic became painless and regular, the hair on the pubes and axillae growing in luxuriantly, while the scalp hair became more abundant, firmly attached and maintained its dark pigment (fig. 5, 6).

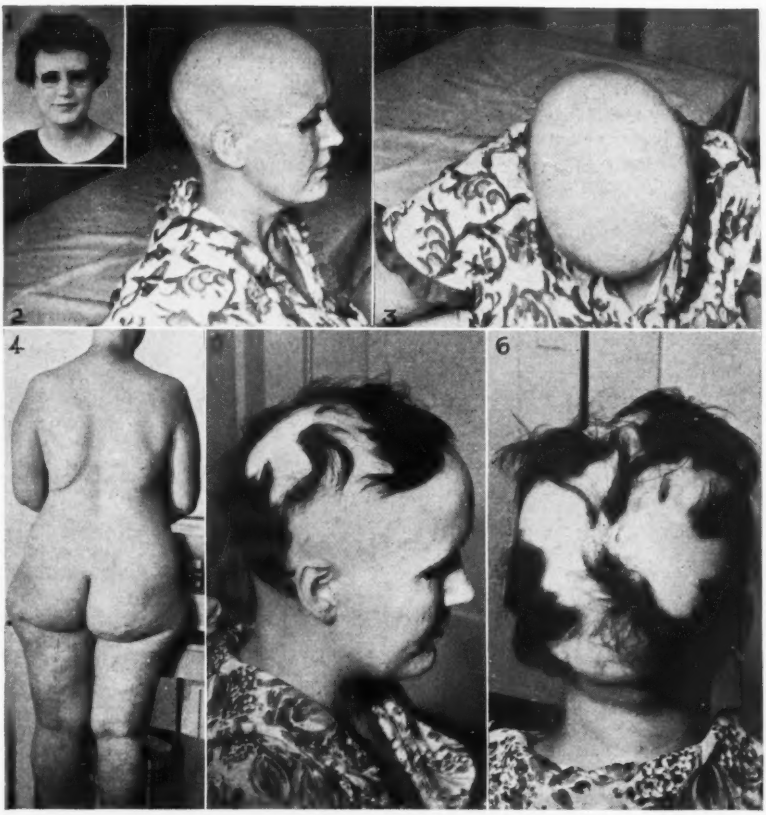

Fig. 1. Photograph taken when only a small area of alopecia had appeared, February 27, 1937. Fig. 2, 3. Appearance of scalp at the time of the patient's first visit in June 1937. Duration of alopecia totalis, 5 months. Fig. 4. Patient when first seen in June 1937. Fig. 5, 6. Scalp at end of therapy with progynon, and one year following anterior pituitary treatment.

Her last menstrual period was in April, being painless but still quite scanty. At the time of this writing (July 12) she is again 3 months pregnant, suffering with nausea and vomiting which has been controlled with crystalline vitamin B_1. Further endocrine therapy is being withheld for the same reason as with her previous pregnancy.

DISCUSSION

Gillespie's report (8) on 3 siblings out of 5 who suffered with congenital alopecia totalis brings further attention to the familial trait in this condition.

The striking features in the case outlined here seem to be the marked endocrinopathic tendencies manifested by 4 members of this family, as well as a definite pituitary-gonadal imbalance in the patient herself, as evidenced by the scanty dysmenorrheic menses, the obesity which began at the menarche and was localized to the trochanteric and iliac crest areas, and the relief from dysmenorrhea experienced following estrogenic therapy.

Whether the anterior pituitary therapy may be credited for having begun the

growth of lanugo and later dark patches of hair over the scalp; whether the subsequent progynon aided in promoting more permanent hirsuties, or whether the present regrowth constitutes merely a spontaneous though temporary restoration of hair which again may weaken and disappear cannot be definitely stated at this time. One can only await further changes in the patient's condition, and hope that her continuous improvement may substantiate the hormonal avenue of approach to the commoner as well as the more unusual forms of alopecia.

Fig. 7, 8, 9. Patient 5 months after writing of paper. Patient has had no further endocrine therapy since onset of pregnancy in April; patient is now 8 months pregnant. Note two remaining small spots of alopecia on which a sprouting of fine lanugo is detected.

From the practical standpoint, it might be suggested here that hormone preparations such as those from the anterior lobe of the hypophysis, which Bengston and Kohn used successfully in some instances, be employed prophylactically in very early common alopecia and defluvium, and with considerable caution, when and if these substances can be manufactured in such pure and stable form that the present unpleasant and unpredictable side-effects from contaminating 'tropic' factors may be avoided.

SUMMARY

The case of a woman is described presenting an alopecia totalis of 2 years' duration along with a definite familial history of alopecia as well as other outstanding endocrinopathies amongst different members of her immediate family.

Various current theories as to the etiology of alopecia areata, and methods of treatment with some of the hormone preparations are discussed as well as the results with such therapy in this patient after one year's observation following anterior pituitary extract and progynon therapy.

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HEXAMINE INSULIN

JUVENILE TYPE OF DIABETES TREATED WITH HEXAMETHYLENE TETRAMINE INSULIN COMPOUND GIVING RAPID AND SUSTAINED ACTION[1]

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IN A DESCRIPTION of protamine insulin in severe diabetes, juvenile type,[1] attention was called to the possibility of an insulin compound with combined immediate and sustained action. In the course of our investigations, our coworker, Dr. Raymond Warburton, developed a compound of insulin and hexamethylene tetramine using 0.25 grains of hexamine to 1000 units of insulin.

There are two objections common to delayed action insulins in precipitated form, namely: (*a*), On standing, there is a tendency of a precipitate to pack. After long packing, it is difficult to get a homogeneous mixture. (*b*), It is impossible to sterilize a precipitated preparation by the common method of filtration.

We have succeeded in preparing a colorless buffered solution of the hexamethylene tetramine insulin compound with the clarity of standard insulin. This clear solution is referred to as hexamine insulin. This report compares the effect of hexamine insulin with standard insulin and protamine insulin.

Protamine Insulin

The introduction of the protamine preparation of insulin was a tremendous contribution to the treatment of diabetes. The practicality of a single daily injection encouraged both the patient and the physician to favor insulin therapy.

There has been some hesitancy on the part of the medical profession to fully utilize this new therapeutic factor because of the following reasons (2).

(*a*), *Allergic risk*. Such reactions have occurred and are more common with protamine insulin than with standard insulin.

(*b*), *Delayed response*. On the basis of the average, as shown on the graph, there is an immediate response in a lowering of the blood sugar level with standard insulin. With protamine insulin, the response is not evident until the 6th hour.

(*c*), *Intercipient dose—Insulin shock*. The rise of blood sugar caused by breakfast is not checked by protamine insulin because of its delayed action. Most investigators found it necessary to use intercipient doses of standard insulin to check this early rise. This often resulted in a summation effect expressed in insulin shock.

Attempts to lower the average blood sugar levels by increasing the dose of protamine insulin (because of the sine-like character of the blood sugar curve) also lowers the low point and results in hypoglycemia.

(*d*), *Precipitate*. The precipitate, on standing, packs and it is only with difficulty that it can be shaken into a homogeneous mixture. Strengths higher than U-80 clog the 27-gauge needle and larger needles are objectionable. A clear solution can be used in any concentration.

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[1] The insulin compound used was prepared and standardized at and distributed from the Metabolic Laboratory of the Kings County Hospital.

(*e*), *Local irritative response.* Annoying local reactions appear at the site of injection of more than 1 cc. of protamine insulin. They are in the form of hard, firm and inelastic nodules which persist two or three days and are gradually absorbed without discoloration (4).

(*f*), *Character of shock.* Shocks caused by protamine insulin, as compared with those produced by standard insulin, last longer and do not respond as quickly to the ingestion of carbohydrates.

Diet

The diets in all cases conformed to the habits of the family and a high carbohydrate-fat ratio was favored. The carbohydrate ingestion was divided as follows: ⅓ at breakfast, ½ at lunch and ⅙ at dinner. Fats and proteins at dinner conformed to the routine habits of the individual patient.

Insulin Shock

With standard insulin, shock occurs 3 to 4 hours after injection of overdose and is rapidly relieved by ingestion of small amounts of carbohydrates.

With protamine insulin, shock is evident 18 to 24 hours after injection of an overdose. The reaction is of the same severity as reaction to standard insulin but lasts longer and requires carbohydrates at repeated intervals and for longer periods.

With hexamine insulin, 6 patients complained of shock 4 hours after injection of overdose, 4 patients 8 hours after injection and 2 patients from 12 to 14 hours after injection. Shock in all cases was immediately relieved by ingestion of small amounts of carbohydrates.

Ketonuria

The absence of ketonuria with hexamine insulin is as striking as with the use of protamine insulin.

CASE REPORTS

Out of 1000 insulin-treated diabetic patients attending the diabetic clinic of the Kings County Hospital, 14 active, most severe cases, were selected for this study.

The dosage of insulin and diet which we have found necessary to maintain this type in a satisfactory mental and physical condition, was used as a criterion for the comparative study. In each case hexamine insulin was administered in a single dose. Blood sugar determinations were made at intervals of 2 and 4 hours throughout the 24 hours, in each case.

Case 1, a white male, age 33, unemployed, height 5 ft. 6 in., weight 150 lb., has been a known diabetic for 4 years. The onset was insidious. Diabetes was mild but became severe in December of 1936 when he was hospitalized for severe hyperesthesia. The diet contained carbohydrates 200, protein 70, fat 80. The dose of standard insulin was 35–35–35. The dose of protamine insulin was 65 U. On February 13, hexamine insulin therapy was established with 65 U daily. On February 27, diabetes was adequately controlled; hyperesthesia improved. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	540	85
Protamine insulin	325	50
Hexamine insulin	200	47

Case 2, a white female, age 17, schoolgirl, height 5 ft., weight 127 lb., has been a known diabetic for 9 years. Onset immediately followed severe whooping cough. The results of a physical examination were essentially negative except for irregular menstruation, periods occurring at intervals of from 1 to 6 months. The diet contained carbohydrates 160, protein 60, fat 30. The dose of standard insulin was 60–40–50. The patient showed persistent aceto-

nuria and glycosuria. The dose of protamine insulin was 150 U. Glycosuria was constant between 8:00 A.M. and 11:00 A.M. There was no acetonuria for a time and then the patient developed an episode of coma of inexplicable origin. On February 13, hexamine insulin therapy was established, with 200 U daily. Sugar ranged from 0.5% to 1% between 8:00 A.M. and noon. On May 27, the patient was comfortable and there was no exacerbation of the diabetic state; this is the longest period she has been free of diabetic crises. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	430	45
Protamine insulin	250	95
Hexamine insulin	300	60

Case 3, a white male age 50, unemployed, height 5 ft. 6 in., weight 145 lb., has been a known diabetic for 6 years. He was admitted to the hospital in diabetic coma in 1931. At that time and since, he has complained of severe headaches. The results of physical examination were negative except for gluteal muscle atrophy on the right side. The patient was treated with standard insulin, crystalline insulin and protamine insulin. The diet contained carbohydrates 220, protein 80, fat 80. Dose of standard insulin was 30–0–15. The dose of protamine insulin was 50 U daily. Headaches persisted. On February 13, hexamine insulin therapy was established with 50 U daily. Headaches cleared. The patient was given protamine insulin for 2 weeks and headaches recurred. On May 20, hexamine insulin therapy was re-instituted and headaches cleared. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	340	105
Protamine insulin	300	62
Hexamine insulin	200	105

Case 4, a white male, age 53, unemployed, height 5 ft. 8 in., weight 154 lb., has been a known diabetic for 10 years. The diet contained carbohydrates 150, protein 70, fat 90. Dose of standard insulin was 60–0–40. Morning blood sugar was 400 mg. Dose of protamine insulin was 95 U. Morning blood sugar was 400 mg. On March 13, hexamine insulin therapy was established. Morning blood sugar was 270 mg. For 2 weeks in April the patient took protamine insulin, 95 U. During this period he lost 15 lb. a week Examination by chemical and biologic assay revealed that the brand of insulin he was using was 20% less potent than the brand on which he had been previously stabilized. On April 21, hexamine insulin therapy was re-instituted. Blood sugar levels in comparative examinations were as follows.

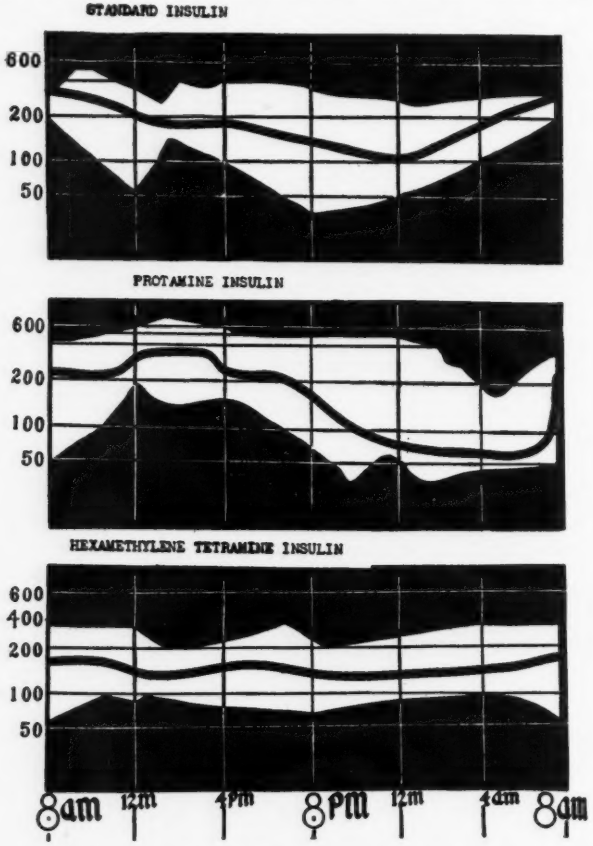
	High level	*Low level*
Standard insulin	440	32
Protamine insulin	420	90
Hexamine insulin	290	130

Case 5, a white male, age 44, watchman, height 5 ft. 6 in., weight 117 lb., has been a known diabetic for 12 years. The diet contained carbohydrates 200, protein 85, fat 125. Dose of standard insulin was 25–20–25. Dose of protamine insulin was 60 U. On February 13, hexamine insulin therapy was established, with 78 U daily. In 3 weeks the patient gained 10 lb. Glycosuria cleared except between 6:00 A.M. and 10:00 A.M. The patient developed frequent mild insulin shocks 8 hours after injection. On May 30, the patient felt well and maintained gain in weight.

	High level	*Low level*
Standard insulin	400	46
Protamine insulin	600	40
Hexamine insulin	190	60

Case 6, a white female, age 25, housekeeper, height 5 ft. 3 in., weight 115 lb., has been a known diabetic for 1½ years. Diabetes developed during an acute stage of hemolytic streptococcus infection (positive blood cultures on 4 occasions). At the same time she developed psychic symptoms suggestive of frontal lobe involvement. The diet contained carbohydrates 150, protein 70, fat 80. The dose of standard insulin was 10–0–10. Although

the patient required little insulin the diabetes was of the severe juvenile type. She was hospitalized for diabetic coma twice during the period of observation. The dose of protamine insulin was 20 U and the diabetic condition remained unchanged under such therapy. On



STANDARD INSULIN CURVE. *A. Average Level.* An average of 12 cases shows a drop of 200 mg. in blood sugar 4 hours after the first injection and another drop of 200 mg. 6 hours after the second injection. *B. Lability of Individual Blood Sugar Levels.* Mention has been made of the marked lability of blood sugar levels in the individual cases. This is shown most clearly in the case of standard insulin where the smallest fluctuation in the individual blood sugar levels is 200 mg. and the greatest 370 mg.

PROTAMINE INSULIN CURVE. *A. Average Level.* Protamine insulin shows a low morning peak of 120 mg. at 6:00 A.M. and blood sugar levels of 230 and 250 mg. at 8:00 A.M. The first 8 hours after injection are characterized by high sustained levels, the average being 350 mg. *B. Lability of Individual Blood Sugar Levels.* Protamine insulin shows almost the same average degree of variability between the individual blood sugar levels as 2 doses of standard insulin but with the maximum variation increased to 450 mg.

HEXAMINE INSULIN CURVE. *A. Average Level.* The average morning blood sugar level is 180 mg. In general the average curve fluctuates between 140 and 170 mg. for the whole day. *B. Lability of Individual Blood Sugar Levels.* The minimum variation between individual blood sugar levels is 105 mg. and the maximum 300 mg.

Clear hexamine insulin has approximately the same action on blood sugar as cloudy hexamine insulin.

April 5, hexamine insulin therapy was established with 20 U daily. Psychic disturbances persisted to a mild degree but there was decided subjective improvement. On June 14, the dose of hexamine insulin was reduced from 20 to 18 U a day because the blood sugar

level with the former dosage was below 100 mg. for 24 hours. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	300	60
Protamine insulin	350	120
Hexamine insulin	100	66

Case 7, a white male, age 35, travelling salesman, height 5 ft. 8 in., weight 170 lb., has been a known diabetic for 9 years. The onset was insidious. The diet contained carbohydrates 300, protein 70, fat 80. The dose of insulin (standard) was 48–0–45. The dose of protamine insulin was 85 U. On March 19, hexamine insulin therapy was established with 96 U daily. The patient was more active and mentally more alert. On May 31, the patient was comfortable and had no complaints. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	440	74
Protamine insulin	440	48
Hexamine insulin	370	250

Case 8, a white male, age 38, candy salesman, height 5 ft. 5 in., weight 142 lb , has been a known diabetic for 7 years. The diet contained carbohydrates 122, protein 80, fat 100. The dose of standard insulin was 25–0–25. The dose of protamine insulin was 50 U. Injection of protamine insulin produced local areas of induration. On March 30, hexamine insulin therapy was established with 50 U daily. The patient complained of insulin shock which occurred 8 hours after injection, especially when he delayed his lunch. On May 27, the patient was comfortable and had no complaints. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	370	100
Protamine insulin	350	70
Hexamine insulin	205	80

Case 9, a white female, age 17½, housewife, height 5 ft. 4 in., weight 163 lb., has been a known diabetic for 3½ years. At the age of 16½ the patient had a normal delivery of a healthy child after 37 hours of labor (3). The diet contained carbohydrates 130, protein 60, fat 50. The dose of standard insulin was 60–0–40. The dose of protamine insulin was 120 U. On March 4, hexamine insulin therapy was established with 120 U and diabetes was adequately controlled. On May 27, the patient complained of nausea, vomiting, headache and severe hiccups with oliguria, ketonuria and glycosuria. She was given 240 U of insulin but the symptoms persisted. Fourteen hours later the blood sugar was 50 mg. and symptoms persisted unabated. She was then given 3 minims posterior pituitary extract and there was complete relief of symptoms in 90 minutes. On May 30, the patient was comfortable and had no complaints. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	375	48
Hexamine insulin	320	54

Case 10, a white male, age 20, office worker, height 5 ft. 11 in., weight 165 lb., has been a known diabetic for 4 years. The diet contained carbohydrates 330, protein 95, fat 80. The dose of standard insulin was 60–0–60. The dose of protamine insulin was 95 U. On April 16, hexamine insulin therapy was established with 110 U daily. On May 28, the patient was comfortable and had no complaints. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	400	110
Protamine insulin	700	235
Hexamine insulin	240	116

Case 11, a white male, age 24, chemist, height 6 ft., weight 155 lb., has been a known diabetic for 8 years. The diet contained carbohydrates 390, protein 85, fat 85. The dose of

standard insulin was 50–0–45. The dose of protamine insulin was 80 U. Polyuria was persistent under standard insulin therapy and protamine insulin therapy. On April 22, hexamine insulin therapy was established with 95 U daily. An May 2, overdose of hexamine insulin (140 U) was given. Marked sweating and severe tremor occurred 3 hours after injection. Seven hours later there was the same sweating and palpitation; pulse was 140. Glucose, 200 gm., gave marked relief in 15 minutes. The residual symptom of instability lasted 24 hours. Thirteen hours after injection there was another, milder shock with tremor and sweating. Glucose, 50 gm., gave immediate relief. The carbohydrate intake for this period was 835 gm.; total caloric intake was 4240. The overdose was repeated on the following day and there was recurrence of the clinical picture above described. The patient was stabilized on the original diet with 70 U of hexamine insulin daily and was comfortable. Daily urinary output under standard insulin therapy was 2200 cc., under protamine insulin therapy 5000 cc., under hexamine insulin therapy 1800 cc. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin	385	120
Protamine insulin	600	40 (shock)
Hexamine insulin	225	100

Case 12, a white male, age 32, physician, height 5 ft. 6 in., weight 189 lb., has been a known diabetic for 10 years. The onset was insidious. The diet contained carbohydrates 280, protein 95, fat 95. The patient took 40 U protamine insulin in the morning, 40 U standard insulin at noon and 40 U standard insulin in the evening. On this regimen the patient complained of hunger, thirst, asthenia, polyuria and pain and muscular twitching in both legs. On April 17, hexamine insulin therapy was established with 120 U daily. Symptoms referable to high sugar levels were relieved. On May 30, the patient was comfortable and had no complaints. Blood sugar levels in comparative examinations were as follows.

	High level	*Low level*
Standard insulin and protamine insulin	280	68
Hexamine insulin	215	85

This patient is now (4/21/39) using 90 units hexamine insulin daily with marked improvement in diabetic state. He has been taking the hexamine insulin daily for 2 years.

Case 13, a white male, age 40, clergyman, height 5 ft. 4 in., weight 168 lb., has been a known diabetic for 14 years. The onset was insidious. The diet contained carbohydrates 150, protein 70, fat 80. The dose of standard insulin was 15–0–15. Morning blood sugar was 400 mg. On repeated examinations there was 3 plus acetone and 4 plus glycosuria at 3:00 P.M. The dose of protamine insulin was 40 U. Morning blood sugar was 200 mg.; at 3:00 P.M. there was 1 plus acetone, 3 plus glycosuria and blood sugar was 400 mg. On April 15, hexamine insulin therapy was established with 35 U daily. Morning blood sugar was 250 mg. At 3:00 P.M. blood sugar was 140 mg. and there was no acetonuria or glycosuria On April 28, diabetes was adequately controlled and the patient felt well.

Case 14, a white male, age 70, unemployed, height 5 ft., weight 100 lb., has been a known diabetic for 22 years. The diet contained carbohydrates 150, protein 60, fat 50. For intervals during 1931, 1935 and 1937, the patient was given 10 U standard insulin daily. Allergic reactions were so severe that the patient refused to continue taking insulin. During February and March of 1937, the patient was given protamine insulin. There was a severe allergic response associated with itch and generalized urticaria. Protamine insulin therapy was discontinued. On April 20, hexamine insulin therapy was established with 10 U daily. There was no allergic response. On May 30, the patient felt well and there was no allergic reaction to the injection of hexamine insulin.

DISCUSSION

The cases here presented are the most severe and difficult cases under our observation. It is of interest to note how many of these patients have relatively high blood sugar levels.

It was at these levels that they were subjectively and physically normal. Frequent attempts to reduce these levels resulted in impairment of activity and subjective distress.

The senior writer has observed hyperglycemic states that persisted over a period of 15 years with blood sugar peaks between 300 and 500 mg. and has observed no evidence that hyperglycemia accelerates degenerative processes.

The average blood sugar level with the hexamine insulin approximates a more normal value in contrast to the variable levels obtained with standard and protamine insulin of the same dose, using a minimum number of injections (fig. 1). The graphs also illustrate that 1 dose of hexamine insulin produces a curve similar to the one produced by many doses of standard insulin but with decided constriction in the range fluctuations of blood sugar.

The graphs have a logarithmic scale. Due to the importance of the variation in blood sugar levels at the range of 50 to 200 mg., a logarithmic representation has been utilized to indicate the relative value of this portion of the blood sugar curve. This representation keeps the percentage change in blood sugar per unit time constant. For example, a blood sugar change from 50 to 100 mg. (a 50% change) is deserving of twice as much space on the graph as a variation from 300 to 400 mg. (a 25% change). A variation of 50 to 100 mg. is given the same space on the graph as a variation between 100 and 200 mg. both being 50% changes.

SUMMARY

A new, clear insulin compound with both immediate and sustained effect is presented.[2]

A single dose may be substituted for 2 to 4 divided doses of standard insulin. One dose of this new insulin produces a curve similar to the one produced by 4 divided doses of standard insulin but with decided constriction in the range fluctuations of blood sugar.

The character of shock experienced with this preparation is comparable to that of standard insulin and responds promptly to the ingestion of carbohydrates.

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[2] This product is not commercially available due to problems of stability.

THE INCREASED DURATION OF INSULIN ACTION BY THE USE OF PROTAMINE ZINC INSULIN IN PELLET FORM[1]

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BEST (1) has reviewed the numerous attempts to prolong the action of insulin. Many recent experiments have shown the increased effectiveness of compressed pellets of estrone, testosterone propionate and desoxycorticosterone implanted subcutaneously (2, 3). We are reporting experiments on the effect of compressed subcutaneous pellets of crystalline Zn insulin[3] and mixtures of crystalline Zn insulin with protamine, on the blood sugar concentration of dogs.

EXPERIMENTAL METHODS

Adult dogs were depancreatized under nembutal anesthesia, and stabilized on a diet of lean meat 250 gm., cane sugar 25 gm., and pancreas 50 gm., given at 9 A.M. and 4 P.M. daily. The dogs received 10 U of insulin daily, except on the day before each experiment.

Pellets were made of crystalline Zn insulin and carefully ground mixtures of Zn insulin and protamine, in an apparatus made for this purpose. The apparatus has two steel plates (fig. 1) with opposing surfaces that are machine ground. These can be fastened firmly together. The upper plate has a number of holes which are 0.5 cm. deep and 0.13 cm. in diameter on the top surface, and very slightly larger on the machine ground surface. Finely powdered crystalline material is scraped into the holes with a spatula, and compressed by hand with a tightly fitting piston until the holes are completely filled. The final compression is made by hammering the piston into each hole with at least six strong blows. The plates are then separated, and the pellets are pushed out by a sharp blow of the hammer on the piston. Pellets were inserted subcutaneously, either dorsally or on the surface of the abdomen with a large needle and a tightly fitting stylet.

Blood sugar determinations were carried out on 2 cc. samples of venous blood according to the method of Folin. Initial blood sugars were taken while the dogs were fasting. The dogs were fed after receiving the insulin preparation. Determinations were made on *dogs B* and *C* before and after pancreatectomy.

RESULTS

The injections of solution of crystalline Zn insulin and protamine Zn insulin produced the characteristic response in depancreatized *dog A* as described by Kerr and Best (4). The Zn insulin solution caused a rapid fall in blood sugar level and a return to a high level in 6 hours. Protamine Zn insulin solution showed a characteristically delayed onset; the blood sugar level fell below 100 mg.% in 12 hours and remained low for 24 hours as shown in figure 2.

Pellets were then made from the precipitate of commercial protamine Zn insulin

[1] Supported by a grant from the Jane Coffin Childs Fund for Cancer Research.
[2] National Research Council Fellow in Pathology.
[3] Supplied by Eli Lilly & Co.

solution. The solution was centrifuged, the supernatant fluid poured off and the precipitate dried in a desiccator, under reduced pressure, over P_2O_5 at room temperature. The residue was then pounded into pellets. These in two depancreatized and one normal dog produced no appreciable lowering of blood sugar, and 48 hours after implantation, exploration of the sites revealed no pellets. The supernatant fluid was also inactive. However, a solution of one such pellet in water produced hypoglycemic action. The exact nature of the change which occurs during dehydration is not clear; it may involve denaturation of the proteins.

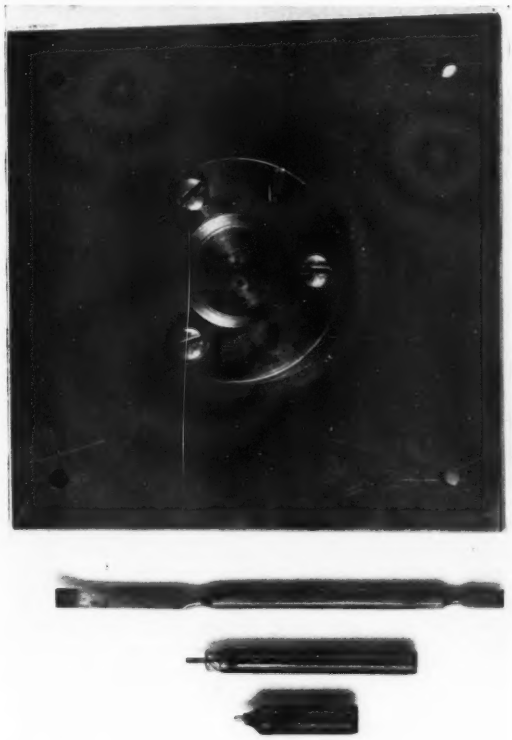

Fig. 1. Pellet-making apparatus.

Pellets made of crystalline Zn insulin and inserted subcutaneously showed marked hypoglycemic action after 4 hours. In *dog C* (intact) a 4.5 mg. (22 U/mg.) pellet produced hypoglycemic shock in 6 hours; in *dog B* (depancreatized) a 4.8 mg. pellet lowered the blood sugar from 268 to 84 mg.% in 4 hours, and the blood sugar reached a high level 24 hours after injection.

A mixture of crystalline Zn insulin 4 parts, with protamine 1 part, the same ratio that is found in the commercial solution, produced the most prolonged lowering of blood sugar concentration in two depancreatized dogs. The fall of blood sugar level below 100 mg.% took place 10 to 28 hours after implantation of the pellet and was maintained below 100 mg.% for approximately 44 to 100 hours. This response was

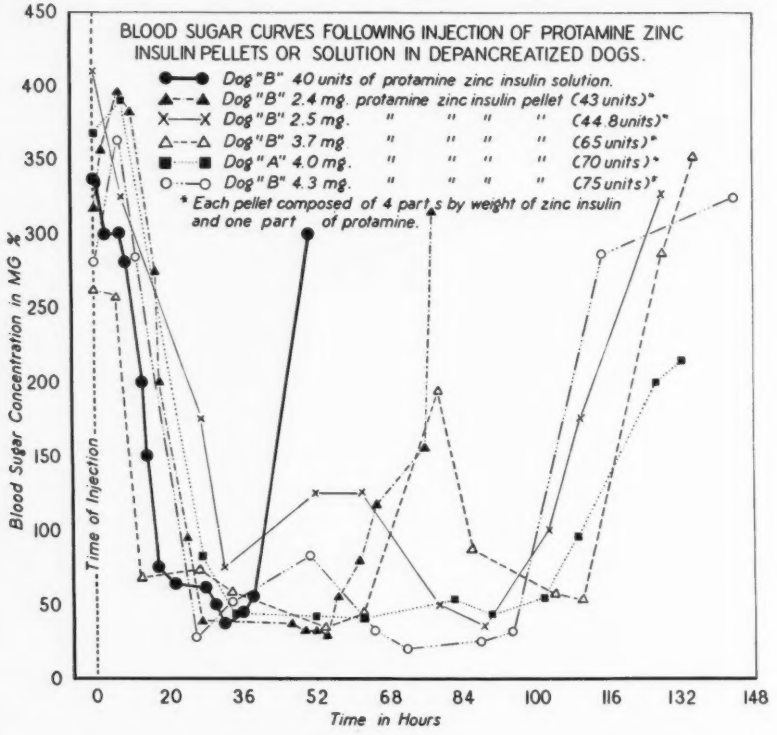
BLOOD SUGAR CURVES FOLLOWING INJECTION OF PROTAMINE ZINC INSULIN PELLETS OR SOLUTION IN DEPANCREATIZED DOGS.
Dog "B" 40 units of protamine zinc insulin solution.
Dog "B" 2.4 mg. protamine zinc insulin pellet (43 units)*
Dog "B" 2.5 mg. " " " " (44.8 units)*
Dog "B" 3.7 mg. " " " " (65 units)*
Dog "A" 4.0 mg. " " " " (70 units)*
Dog "B" 4.3 mg. " " " " (75 units)*
* Each pellet composed of 4 parts by weight of zinc insulin and one part of protamine.
Blood Sugar Concentration in MG %
Time of Injection
Time in Hours
0
50
100
150
200
250
300
350
400
450
0
20
36
52
68
84
100
116
132
148

Fig. 2.

obtained 4 times in *dog B* (depancreatized) and once in *dog A* (depancreatized), as illustrated in figure 2. Pellets of this mixture containing 43, 44.8, 65, 70 and 75 U of insulin were used. A pellet with 44.8 U of insulin maintained a blood sugar level below 100 mg.% for 72 hours; one with 86 U caused hypoglycemic shock after 48 hours. Another pellet of 43 U in this same dog produced a blood sugar level below 100 mg.% for only 44 hours. We can not account for these variations from our experiments. The relatively few observations, the inability to secure the same degree of compactness of the pellets, and possible local differences at the sites of inoculation may account for these variations.

A pellet prepared of equal parts of protamine and Zn insulin containing 37 U, caused a lowering of the blood sugar level for 4 hours in *dog C* after pancreatectomy. A similar pellet with 41 U in *dog B* before pancreatectomy had a slightly longer duration of action. Two larger pellets were made of 4 parts of insulin and 1 part of protamine containing 261 U and 1,012 U of insulin, respectively, to give a much larger surface area. These produced a fall in blood sugar level below 100 mg.% in 5 hours, and hypoglycemic shock within 24 hours. The site of implantation in both cases was yellowish and extremely edematous over an area of 3 to 4 cm. The pellets had lost their form and varying sized granules, from pin-head to match-head sizes, were found. Microscopic examination of the tissues from these areas showed extreme edema at the sites where the pellets were introduced with profuse polymorphonuclear infiltration. Bacterial stains showed no evidence of organisms.

DISCUSSION

By using a mixture of 4 parts of crystalline Zn insulin and 1 part of protamine in pellet form, it is possible to maintain a blood sugar level below 100 mg.% in depancreatized dogs for 44 to 100 hours with a single injection. Pellets containing 65 to 75 U of insulin were most effective; smaller pellets were effective for shorter periods; a larger pellet produced hypoglycemic shock. The onset of marked action of the 4:1 pellet occurs after 10 hours with small pellets, but occurs in 5 hours when large pellets are exposed to tissue fluids. Zn insulin solutions produce rapid effects and Zn insulin pellets are slightly slower in the onset of their action. The efficacy of the pellet is probably due to the slow absorption of the insulin from the tissues at the site of inoculation. However, since the Zn insulin pellet has so little superiority over the Zn insulin solution, the addition of protamine in solid form must be a decisive factor. Hagedorn (5) found that mixtures of protamine and Zn insulin in a 5:1 ratio produced iso-electric insulin buffered at the PH of body fluids. This reaction probably also takes place with the solid materials in the tissues. Possibly the protamine present in the 4:1 mixture gives a more alkaline insulin compound in the tissues, which may be more soluble.

Protamine injected into animal tissues produces a marked inflammatory reaction. However, this local reaction can not be of great importance in causing a prolonged hypoglycemic action, since the 50:50 mixtures were so ineffective.

Since the local reaction and suitable PH, both possible with protamine in the commercial solution, produce a hypoglycemic action for only 24 hours, there must be additional factors at work with the solid mixtures. From our experience with estrone and testosterone propionate pellets we felt that pellets of insulin might produce a greatly prolonged action. More rapid absorption would be expected from the water-soluble insulin than from the fat-soluble sex hormones. Six and one-half hours after implantation we examined a pellet of 4 parts of insulin and 1 part of protamine and found marked edema of the site and swelling of the pellet, with no lowering of the blood sugar and no lowering occurred after its removal. Absorption must take place from the surface, but not in sufficient concentration to show itself in the blood sugar level with pellets of 2.4 to 4.3 mg. in this period of time. The two large pellets of a 4:1 mixture containing 261 and 1,012 U of insulin, with much larger surface areas, produced a low level in 5 hours. These were not examined at this time.

Since the hypoglycemic action persisted for 44 to 100 hours below 100 mg.%, perhaps the absorption from the larger granules present after the pellet fragments, allowed slower absorption than that which occurs from the finely divided particles in the protamine Zn insulin suspensions. This is one difference which may account for the delay in absorption.

SUMMARY

Pellets prepared by compressing a mixture of 4 parts of crystalline zinc insulin and 1 part of protamine, containing the equivalent of 43 to 88 U of insulin, were administered subcutaneously to depancreatized dogs. These pellets produced a marked lowering of the blood sugar concentration for from 44 to 100 hours. Two larger pellets of this mixture, containing 261 and 1012 U, respectively, caused a rapid fall in the blood sugar concentration and hypoglycemic shock within 24 hours. Pellets of crystalline zinc insulin did not show a prolongation of hypoglycemic action. Pellets prepared from a mixture of equal parts of crystalline zinc insulin and protamine were not as effective in lowering the blood sugar concentration as the pellets prepared from the 4:1 mixture. The precipitate of commercial protamine zinc insulin was centrifuged, dried and compressed into a pellet; but these were inactive. When this type

of pellet was redissolved, the solution showed hypoglycemic activity. The active pellets of the 4:1 mixture produced a marked local edema at the site of implantation. The two larger pellets fragmented as they were absorbed.

We are grateful to Dr. W. G. MacCallum and Dr. Barnett Cohen for their advice and kind interest in this problem.

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THE EFFECT OF TESTOSTERONE ON THE MONKEY UTERUS AND THE ADMINISTRATION OF STEROIDAL HORMONES[1] IN THE FORM OF DEANESLY-PARKES PELLETS

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THE PURPOSES of the present investigation were twofold: (*a*) to compare the action of the hormone when compressed into hard pellets and placed under the skin with the same hormone administered by the more common method of injection in oil; (*b*) to determine the effect of androgens on the endometrium of mature rhesus monkeys under various conditions.

PART I. OBSERVATIONS ON THE USE OF DEANESLY-PARKES PELLETS

At the colloquium on the physiology of reproduction held in Paris in 1937 under the auspices of the Singer-Polignac Foundation, Dr. Ruth Deanesly (1) reported on various methods employed by her to attain a continuous and prolonged, hence nearly natural physiological action of the fat-soluble hormones. Among these methods was included the direct implantation of the crystalline products. Dr. Geschickter and the writer (2) had also previously used large crystals of estrone with some success. Deanesly found, however, that if the crystals were pressed together into a hard pellet or tablet before implantation into the organism, the action of the hormone was greatly prolonged. Upon his return the writer introduced the method in Baltimore where the results have been gratifying (3, 4) and fully corroborative of the work reported from the Institute for Medical Research, London.

Preparation and Implantation of Pellets

Because of the high potency of the sex hormones, especially the estrogens, it seemed desirable to prepare small pellets which could be readily introduced under the skin by means of a trocar. To prepare these the apparatus shown in figure 1 was devised. It consists of two plates of steel with carefully lapped faces and is provided with 3 machine screws for fastening the plates firmly together when in use. The upper plate has a depression $\frac{1}{8}$ inch deep in the bottom of which 16 holes are drilled with a #60 drill and reamed out so as to flare slightly downward in order to facilitate removal of the finished pellets. The reaming is, however, not indispensable.

To prepare the pellets the hormone crystals are squeezed into the holes by means of a steel spatula and then driven in lightly by means of the 'pile driver' device shown in the illustration. This consists essentially of a shaft into the bottom of which a short

[1] The writer wishes to acknowledge the generosity of the following firms: E. R. Squibb and Sons for amniotin Squibb (estrogens, chiefly estrone) furnished over a period of years; Schering Corporation for crystalline estradiol as well as estradiol benzoate (progynon-B, Schering) in oil, also progesterone (proluton, Schering); Ciba Pharmaceutical Products, Inc., for crystalline testosterone acetate and propionate; Parke, Davis & Co. for crystalline estrone (theelin).

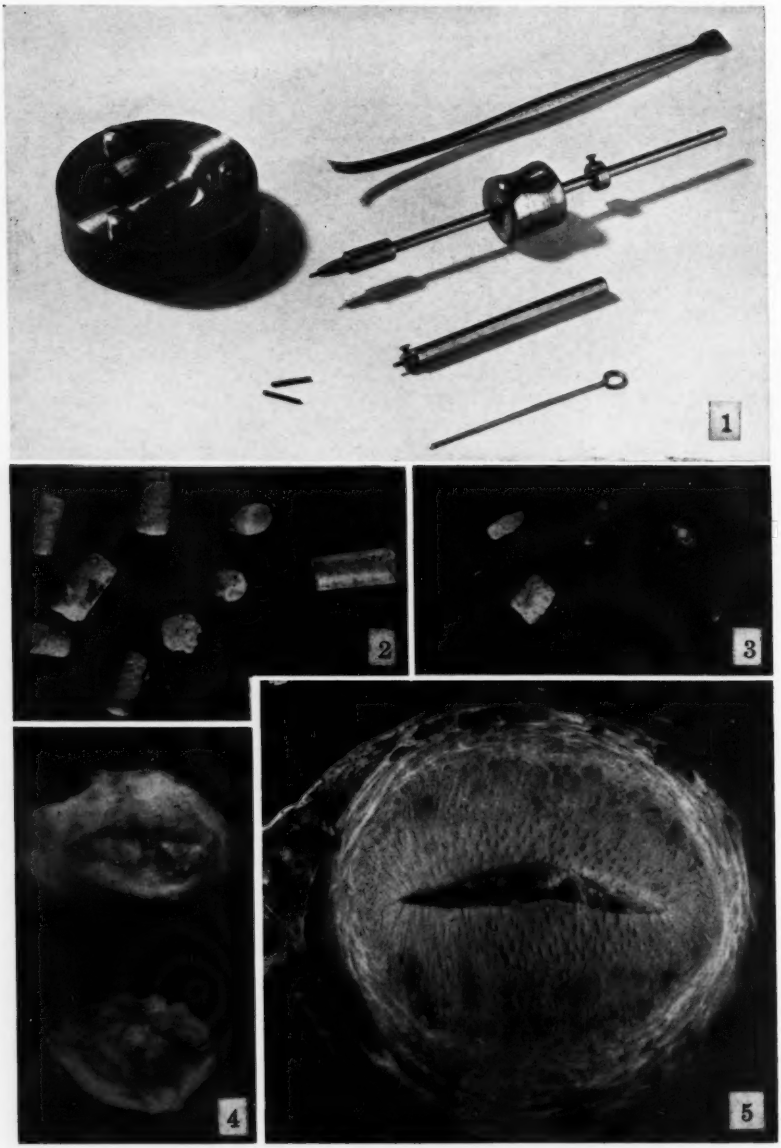

PLATE I., Fig. 1. HORMONE PELLET APPARATUS DESCRIBED IN TEXT. Fig. 2. PELLETS REMOVED FROM NO. 544, WITH ONE UNUSED PELLET AT RIGHT (×5). Fig. 3. REMAINS OF PELLETS FROM NO. 573, (×5). Fig. 4. ENCAPSULATED PELLETS FROM NO. 569, CHART 1, (×4). Fig. 5. THICK SECTION OF UTERUS OF NO. 576, IN ALCOHOL, UNSTAINED (×4).

piece of tempered #60 stainless steel wire may be fitted and renewed as needed. Tamping of the accumulated hormones is accomplished by dropping the weight. When the holes have thus been filled to overflowing the crystals must be further compressed to make the pellet hard and firm. In the absence of a hydraulic press the writer used a hammer applied to a simple tamper (fig. 1), a short octagonal rod provided at the bottom with a piece of #60 tempered wire, readily renewable when buckled by use. A good pellet has a smooth shiny surface and will not readily shatter, nor should it crumble when put in place beneath the skin.

The pellets made with this apparatus weigh just under 3 mg. for each 2 mm. of length. One properly made pellet of theelin or progynon-B may prevent castration changes in a monkey for 4 months.

The placing of the pellets under the skin is simplicity itself. One or more of them are laid in a suitable trocar sharp enough to perforate the skin with a quick stab. Once the skin is pierced the pellets are pushed out with a plunger and the trocar removed. The trocar is, of course, sterilized. The pellets used were not sterilized, although every precaution was taken to reduce the possibilities of contamination. The pellets may, of course, be heat sterilized, due attention being given to the melting point of the compound.

Local Tissue Reactions

Due to ordinary precautionary measures infections were never encountered although the monkey is readily susceptible to skin infections. The pellets become encapsulated (fig. 4) like any other sterile foreign body, such as a silk thread. In sections connective tissue hypertrophy and infiltration of polymorphs can be seen. The blood supply, as judged by the relative pallor or redness of the lining of the freshly excised capsules, varied considerably, a fact that might account for variation in rate of absorption. No observations were recorded which might point to any differences in tissue reactions toward the several hormones used.

Physiological Action of Hormone Pellets

The intensity and duration of the action of the hormones administered as described above are illustrated by means of the following actual experiments. After presentation of the protocols the results of all of the experiments are summarized.

(*a*) *Inhibition of castration bleeding by theelin pellets.* In all of the 6 animals listed in series *B*, chart 1, the implantation of a single pellet inhibited completely the bleeding that would otherwise have followed castration. Hormonal influence was also reflected in the increase of vaginal desquamation (chart 1). The results in general in this series compare favorably with the 6 other cases listed in series A, chart 1, receiving estrogen by injection. Of the latter 6 cases one *monkey* 393, castrated on day 23 of the menstrual cycle, bled 2 days later.

One further case may be cited, *monkey* 577. This 4510 gm. mature female received 60 units of mare's serum on March 30, a pellet of theelin on April 1; was castrated on April 2. Although one of the ovaries contained a 5 mm. follicle, no bleeding following this castration. Vaginal desquamation and sex color continued high. On April 20 the pellets were removed and bleeding occurred 5 days later (April 25) when the pellets were replaced; this restored the slightly waning sex color and the vaginal desquamation.

Thus, in seven cases one 3-mg. theelin pellet each sufficed to prevent the bleeding which normally follows castration. In *monkey* 577 bleeding occurred 5 days after removal of the pellets. Re-implantation of the pellets restored the estrogenic effects.

(*b*) *Failure to affect uterine bleeding. Monkey* 97. This female was born in the colony on April 8, 1930, grew to maturity and became a much studied individual. Menses were re-

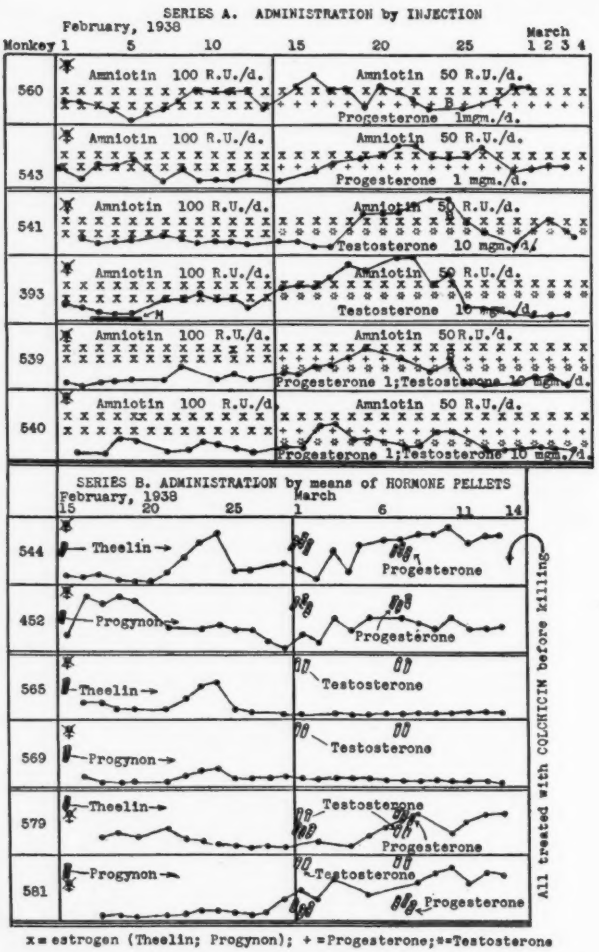

CHART 1. SOME DETAILS CONCERNING 6 EXPERIMENTS WITH HORMONES ADMINISTERED BY INJECTION AND 6 BY IMPLANTATION OF THE SAME HORMONES IN THE FORM OF PELLETS. ALL WERE CASTRATED ON THE DAY TREATMENT BEGAN. M, BLEEDING (No. 393); B, UTERINE BIOPSY (No. 560, 541, 539). CURVES OF AMOUNT OF VAGINAL DESQUAMATION ARE SUPERIMPOSED IN THE CHART.

corded for Feb. 25 and March 21, 1938. On March 30 she received 30 Upjohn units of gonadogen intravenously, on April 3 three 3 mg. pellets of testosterone propionate subcutaneously. Bleeding nevertheless began on April 9 (cycle 20 days) and continued for 8 days. On April 20 the pellets were removed but no bleeding followed within the next month.

Three pellets of testosterone propionate imbedded subcutaneously thus failed to prevent the current menstrual flow, nor did their removal result in further bleeding within the following month.

Monkey 237. This female was received in Oct., 1932, weighing 4720 gm. (4 years later, 5680 gm.). The animal was variously utilized from 1932 to 1936. Ovulation was induced by

gonadogen in Oct., 1937 (5). On March 8, 1938, the animal furnished a 110-day fetus removed by caesarean section. On April 4, 1938, two 3 mg. pellets of progesterone were introduced subcutaneously; there was a very slight bleeding on April 9 and 10. On April 20 the pellets were removed, but there was no bleeding as a consequence.

Two pellets of progesterone did not prevent a slight bleeding in this female, one month after a caesarean operation, nor did the removal of the pellets result in bleeding.

Monkey 516. Twice hysterotomized, this 4800 gm. female was used in experiments with gonadogen on Feb. 4, and on March 1, 1938. Menses occurred on March 14; cycle 20 days in length. On March 31, 3 pellets of testosterone propionate were implanted subcutaneously and the surviving ovary (10 ×8 ×7 mm.) was removed the next day. The hormone did not prevent uterine bleeding which began 2 days after castration. The ovary contained graafian follicles nearly 0.5 mm. in diameter and old corpora albicantia but no current corpus luteum. On April 12 the pellets and their tissue capsules were excised. No bleeding had occurred by April 27, when the animal was killed.

In this case absorption from three 3 mg. pellets of testosterone propionate was too meager to prevent uterine bleeding following castration late in the cycle and also seemed insufficient to bring about 'testosterone deprivation' bleeding following the the removal of the pellets.

(*c*) *Regeneration of endometrium stimulated by hormone pellets.* The endometrium possesses great capacity for regeneration, not only after each menstrual sloughing and after each parturition but also after surgical removal. The writer has excised successively from the same animal as many as 3 implanted embryos with almost the entire maternal endometrium. Several such hysterotomies indeed were practiced in successive months (cycles?). In some instances, here described, the endometrium was not only excised in the usual manner but in addition was wiped as clean as possible with a cotton sponge. Rengeneration, with cell division, occurred in several cases after castration without the administration of hormones but in such cases proliferation progressed very little, if at all, beyond the point of epithelializing the wound surfaces. Beyond this point the growth of the endometrium seemed to require the stimulus of sex hormones.

Monkey 513. This 4750 gm. female ovulated in September and October, 1937; on day 9 of the December cycle a 3 mm. follicle was found in the ovary. The ovary seemed less active in January and February, 1938. hence pregnant mare's serum was injected (5). A cystic ovary was removed on Feb. 9, the surviving ovary on March 18. At the last operation the uterus was opened and the endometrium removed as thoroughly as possible. At this time two 1 mg. pellets of theelin were implanted subcutaneously. The animal was killed 18 days later. The endometrium was completely restored, though rather thin. Prior treatment with colchicine brought out many mitotic figures throughout the mucosa. Regeneration had, however, preceded less than in *monkey 454* that received progesterone also or in *monkey 401* which had ovulated.

Thus in 18 days the uterine lining was completely restored after complete denudation of the endometrium. Pellets of estrone speeded the restoration as compared with castrates not receiving hormone treatment.

Monkey 454. The first menstrual cycles of the 1936–1937 season were very irregular and were punctuated by long periods of amenorrhea. More regular in the fall of 1937, the female ovulated on Nov. 13–14 and on Dec. 17; menses began on Dec. 31. Daily injections of 10 mg. of testosterone were made Jan. 3–8 inclusive, but were not followed by ovulation as checked by a laparotomy on Jan. 10; the cycle was shortened to 18 days. Bleeding occurred on Feb. 18, followed by a non-ovulatory cycle. Gonadogen injections (5), beginning Feb. 15

(day 2), failed to stimulate the ovaries. Castration was carried out on March 17; also a uterine biopsy and thorough removal of the endometrium by rubbing out with a sponge. At the same time theelin and progesterone pellets from *monkey 544* (see below) were transferred to this animal and thus used a second time. At the beginning of the experiment biopsy showed the endometrium to be in the resting stage (fig. 6); 19 days later the regenerated endometrium had reached the pregravid condition shown in figure 7.

Thus the same pellets of theelin and progesterone which had brought about an early secretory endometrium in *monkey 544* in 13 days, after removal from this animal and implanted into No. *454*, caused the transformation of the completely restored endometrium of this female into a moderately advanced secretory phase in 19 days.

(d) *Dominance of estrin effect.* Although estrone or estradiol are more slowly absorbed than progesterone, the estrogen is more potent in bringing about observable effects in the organism, hence is likely to predominate over the progestin effect. This has already been noted in *monkey 580* cited above. It was also observed in 398 but more especially in *566* which received 2 pellets instead of a single one.

Monkey 398. This mature female, weighing 5570 gm., furnished one embryo Oct. 12, 1936 and another just 2 months later (Dec. 10), when she was castrated. Being used for experiments in sex behavior she received progynon-B injections from time to time, the last series Feb. 7–12, 1938. On March 11 a pellet of theelin was introduced, supplemented 5 days later by pellets that had been removed from No. *452*, mainly progesterone. The sex color became maximally intense and the edema of the sex skin extended in ridges far up the back, which indicated a preponderance of estrin effect. The pellets were removed on Aprli 5, whereupon the sex color and the swellings receded and light bleeding began 3 days later, increasing in amount the next day, when the animal was killed. The endometrium contained many mitoses, the surface epithelijm was thick, as were some glands; other glands showed undoubted progestin influence, especially the basal ones which were convoluted and secretory.

Nineteen days exposure to theelin and progesterone pellets gave a strong estrin, a fair progestin effect. The proportion of the two hormones was not optimal. Excision of the pellets brought on uterine bleeding in 3 days.

Monkey 566. This animal weighed 3960 gm. when received Aug. 3, 1937. The first menstruation was observed on Nov. 29. On Jan. 17, an embryo was recovered and one ovary was removed. March 11–16, she received pregnant mare's serum, but did not ovulate. On March 18, 39 days after the last menstrual period the surviving ovary was removed, the uterus curetted and wiped as free of endometrium as possible. At this time also the animal received the theelin and the testosterone pellets that had been used by Nos. *565* and *569* and in addition thereto three 3 mg. pellets of progesterone. On April 5, 10 hours after colchicine administration, the animal was killed and at autopsy was found to be severely infected with tuberculosis. The endometrium had been restored but only to the thickness of 1.5 mm., without signs of secretion. The entire endometrium was studded with mitotic figures.

Eighteen days action of 3 progesterone pellets failed to produce a pregravid state in the endometrium of this animal, perhaps because of the dominating effect of 2 estrin pellets where one would have been sufficient. It is not likely, in view of other experiments, that the testosterone augmented the antagonistic action of theelin toward progesterone in this case.

(c) *More rapid absorption of progesterone pellets than those of estrone or estradiol.* Each of two animals (*monkeys 580* and *573*) was the third recipient of a batch of pellets. Thus pellets resided successively in females *544*, *454* and *580;* another lot

in 452, 398 and 573. In the case of 573 it was found that, on removal of the encapsulated hormone pellets, remnants of only the estrone pellet remained (fig. 3).

Monkey 580. This 4840 gm. female was subjected to an experiment with mare's serum (5) and then castrated. One ovary contained a 3 mm. follicle. At the time of castration she received pellets of theelin and progesterone which had previously been in *monkey* 454, (fig. 2), for 19 days and before that in 544 for 7 to 13 days. The absorbed hormones all but prevented the usual bleeding following castration, as only a few red blood cells were seen in the vaginal lavage on the second day following the loss of the ovaries.

On June 14, 37 days after introduction of the pellets, these were removed. No bleeding occurred as a consequence of the excision of the hormone pellets, although resorption of material from the pellets was indicated by the brightness of the sex skin and the high vaginal desquamation; the endometrium was low, but the epithelia showed numerous mitoses even without the colchicine technic. There was not the slightest indication of progestin effect.

Thrice transplanted pellets of theelin and progesterone all but prevented the post-castration bleeding but after 37 days the animal showed only theelin response. Removal of the pellets failed to result in bleeding within 10 days.

Monkey 573. This 4640 gm. multipara was used in an experiment with pregnant mare's serum (5) in February, 1938. Bleeding occurred on March 18. On April 6 she received subcutaneously 2 theelin and 6 progesterone pellets that had been removed from animals 566 and 398. The latter pellets had previously been in 452 for 13 days, and in 398 for 19 days. No. 573 was castrated the day after receiving the pellets. No bleeding followed castration, although the ovaries contained a number of follicles up to 2 mm. in diameter. Sex color and vaginal desquamation remained high for one month, then gradually diminished. When the animal was killed (May 25) the sex color was still fair, but the uterus small (14 × 11), the endometrium thin and its epithelia much retrogressed. There was no indication of progesterone effects. Several capsules about the pellets were excised. One of these contained a theelin pellet but the progesterone had been almost completely absorbed (fig. 3).

The thrice transplanted pellets of theelin and progesterone finally received by this female prevented the bleeding of castration but remained effective for somewhat less than a month. At autopsy there was no longer a progestin effect.

(*f*) *Duration of effectiveness of implanted pellets.* In the castrated monkey the sex skin is a delicate indicator of the presence of estrin in the blood. Desquamation of cornified cells from the vaginal wall helps in the diagnosis of estrin activity. By these criteria, as well as others, it was found that a 3 mg. pellet of progynon-B may last as long as 4 months. In a rat, my colleague, Dr. J. Ball, has observed the continuance of low estrogenic activity from a single small pellet for over 6 months. Details are given in the following protocols.

Monkey 282. This very large female weighed 6160 gm. when received Oct. 3, 1933. On Nov. 27 she furnished a 25-day embryo. Succeeding cycles were 25, 26, 29, 28, 27, 29, 27 and 27 days in length. On Feb. 6, 1935, her ovaries and uterus were removed; the uterus contained an 11-day ovum. From 1935 to 1938 she was variously used by Dr. J. Ball in studies on sex behavior in relation to the administration of estrogen (progynon-B, Schering). The last series of injections extended from Jan. 28 to Feb. 19, 1938. On Feb. 22 one 3 mg. pellet of theelin was placed under the skin. Every external sign of estrin influence continued to June 13, that is, nearly 4 months after implantation of the pellets, when the sex skin seemed to possess slightly less than maximal redness. By June 23 the color had paled still more. She was killed on June 24.

Throughout the 4-month period the sex skin maintained a maximal redness. The buttocks were never swollen, which is not surprising, since it requires a high dosage of estrogen to produce sex swellings in old multiparas, which almost never exhibit such edema normally. The vaginal desquamation remained up to 30% the greater part of the time; both cervix

and vagina exhibited every evidence of estrogenic stimulation. The hypophysis was unusually large, as in the pregnant monkey (105 mg.)

One 3 mg. pellet of theelin had a continuous effect upon this large castrated female monkey for a period of 4 months.

Monkey 234. This large multipara (5 kg.) furnished a 146-day fetus on April 27, 1934, a 135-day fetus on April 22, 1936, both removed by caesarean section. On Oct. 27, 1936, she suffered removal of ovaries and uterus; the uterus contained an 11-day ovum. Used in investigations on the relation of hormones to sex behavior she repeatedly received injections of progynon, the last series ending Feb. 19, 1938. On Feb. 22 a 3 mg. pellet of progynon-B was implanted which maintained a red sex color for 2 months. She was killed on April 27 in an advanced state of tuberculosis after having been in the colony for 6 years. The progynon pellets were recovered and introduced into female 580 (see above).

Thus a 3 mg. pellet of progynon sufficed to preserve the sex skin and vagina of this monkey in the estrous state for 2 months. In this interval the pellet suffered only slight reduction in size.

Summary of Experiments with Hormone Pellets

A properly made pellet of fat-soluble hormone is firm and non-friable and presents a smooth, shiny absorption surface. The limited surface thus presented to the tissue juices greatly reduces the rate of absorption of the pellet implanted subcutaneously. The absorption can readily be regulated to approach the physiological in quantity as in continuous action. It is economical of material and especially of time. The results of Deanesly and Parkes (6, 7, 8) are confirmed in every particular.

The method is especially applicable where small amounts of hormone over long periods of time are desired. Physiological effects over a period of 4 months from a single 3 mg. pellet of theelin are recorded. Such a pellet usually inhibits castration bleeding but it may fail to do so even where no current corpus luteum is present in the ablated ovaries. Similarly, removal of the pellets, even where progesterone is included, may or may not result in hormone-deprivation bleeding. These facts argue for a low level of absorption, as one may conclude from the data on hormone dosages necessary to bring about menstruation in the castrated monkey, presented by Corner (9) and Zuckerman (10).

In consonance with the work of other investigators the present series showed progesterone to be more rapidly absorbed than the estrogens employed. However, the tests for the latter are several times more sensitive than the former, hence, weight for weight, several hundred times more progesterone than estrogen must be administered to secure an observable effect on the organs studied.

The slowness of absorption, combined with the continuity of action, of the hormones would seem to render the method advantageous for some clinical situations, chiefly 'chronic' conditions as, for example, menopausal difficulties in which it has already been employed (11). However, where a quick reaction to a large dose or a rhythmicity is required, as for example in amenorrhea, dysmenorrhea or functional bleeding, the method has disadvantages as compared with that of injection or inunction.

Perhaps the greatest drawback to the pellet method of administering hormones to human patients, lies in the remote chance of causing an infection. This problem lies beyond the scope of this paper. The statement may be made, however, that precautions have thus far prevented infection in the monkey in all cases.

Attention should be called to various other means that have been employed to prolong the action of administered hormones. Robson and colleagues (12) were able

to note the effect of a single subcutaneous injection of 500 γ of estradiol benzoate butyrate after nearly 4 months. Engle and Crafts (13) could inhibit menstrual bleeding in a monkey for 2 months with huge doses of various estrogenic compounds. Deanesly and Parkes (7), however, have subjected to careful analysis the various factors making for continuity and prolongation of action of the hormones—subdivision of dose, increased volume of vehicle, addition of fatty acid, esterification, inunction, implantation of crystals, implantation of hard tablets—and have concluded that the last method attains the ends desired in the most satisfactory manner.

PART II. THE EFFECT OF TESTOSTERONE ON THE MONKEY ENDOMETRIUM AS COMPARED WITH ESTRONE AND PROGESTERONE

The present investigation attempts to answer the specific question: what is the effect of testosterone or some of its derivatives on the endometrium of the monkey? Does it, like an estrogen, cause proliferation of the epithelial and connective tissue elements and bring about edema (14, 15, 16)? Or will testosterone produce progestational changes, thus replacing its near relative progesterone (17, 18, 19)? Does testosterone antagonize estrin or cooperate synergistically with it (20)? Does it enhance or inhibit the progestational effect of progesterone or does it do neither? Finally, may testosterone bring about deleterious changes or even atrophy (21, 22)?

The large and growing literature on the physiological effects of testosterone will not be here reviewed, suffice it to say that all of the questions just propounded have been answered by various authors in the affirmative.

The present series of experiments on the monkey endometrium are varied as to dosage, manner of administration, duration of exposure, and kinds of hormones concomitantly injected. The majority of the experiments were made on castrated females; but in view of the possible, indeed quite probable action of injected hormones via the anterior pituitary and ovaries (23, 24), a few experiments have been repeated on intact monkeys. It would seem that the variety of the experiments here recorded offers a rather definite answer to our inquiry, to the effect, namely, that while testosterone may be credited with estrogenic value in that it prevents castration atrophy of the uterus, it has practically no progestational effect. Neither has any indication been observed that testosterone either interferes with or enhances the progestational value of concomitantly administered progesterone.

Experiments on Castrated Female Monkeys

Concerning 12 experiments listed in chart 1. This study was originally planned to include only 12 females that had to be destroyed because of an epidemic of tuberculosis in the Carnegie colony. Several of the subjects were actually badly infected, but the tubercular state did not seem to affect the results appreciably when comparison was made between these and results attained on sound subjects.

The manner of administering the hormones may in each case be read from chart 1, which also shows the curve of vaginal desquamation. The first 6 animals (series *A*) were injected with hormone dissolved in oil, the second series (*B*) were given pellets as described in Part I above. All animals were primed with estrin, as indicated in the chart and had estrin administered throughout the experiment. Two members of each group received progesterone for a period of time before autopsy, 2 received testosterone and two both testosterone and progesterone. The findings in each case may be ascertained from the following protocols.

Monkey 543. This female weighed 3790 gm. when received on April 22, 1937. Cycles 39, 31 and 28 days in length followed, of which only the second was ovulatory. The animal

was castrated on Feb. 2, 1938, day 28 of a non-ovulatory cycle (chart 1, series *B*). The ovaries were small and possessed but a single corpus albicans. The injected progynon prevented the bleeding of castration. The added progesterone (chart 1) in 19 days brought about a typical pregravid endometrium (fig. 13), 5 mm. in thickness in the fresh state. The glands were secretory (fig. 15), the uterine epithelium low, as 14 or more days after ovulation in the normal cycle (fig. 14).

A near-maximal effect was attained with progesterone in this case, which compares favorably with *monkey 540* (fig. 17, 18) which was similarly treated but received testosterone in addition.

Monkey 560. This young nullipara weighed 2580 gm. on May 6, 1937, 3530 gm. on Jan. 31, 1938. The first bleeding observed (Jan. 11) was slight and was accompanied by high vaginal desquamation. The animal was used in series A (chart 1). She was castrated on Feb. 1, day 23 of the cycle. The endometrium remained thin (1.5 mm.) but showed some response to the introduced hormones, namely, slight tortuosity of the glands, distinct evidence of secretion, realignment of gland nuclei and thinning of the surface epithelium. The progressive nature of these changes becomes apparent when one compares the uterine biopsy taken on Feb. 24 (chart 1) with the endometrium of March 4. The animal had abdominal tuberculosis when killed.

The endometrium of the female was the most unresponsive of the 8 animals receiving progesterone, though some evidence of the action of the hormones was present.

Monkey 544. The weight of this young female was 3170 gm. when received on April 22, 1937, 4210 gm. on Feb. 1, 1938. Menses were irregular and no ovulation occurred, though the sex skin was considerably swollen. In February, 1938, the vaginal desquamation was low (chart 1). Ovaries of infantile size were removed on Feb 5, 1938, 37 days after the last menstrual period, whereupon hormone administration was begun as shown in chart 1 (series *B*). At the end of the experiment the uterus measured only 14.5 × 11 mm.; the endometrium, 3 mm. in thickness, was in early secretory phase, with thick pseudostratified uterine and gland epithelium. Mitoses were found throughout the mucosa, brought to light by prior colchicine treatment.

Pellets of progesterone with theelin brought the endometrium of this young non-ovulating animal no further in 13 days than is normally attained by about 8 or 9 days after ovulation in the menstrual cycle.

Monkey 452. The animal weighed 4390 gm. when received on June 13, 1936 There was no ovulation in the season of 1936–1937. On Dec. 14, 1937, an 11-day ovum was recovered. The cycle of Jan. 12 to Feb. 7 was ovulatory. Castration was performed on Feb. 15, day 9 of cycle; the ovaries measured 7.5 × 5.5 × 6.5 and 7 × 5.5 × 4.5 mm. Progynon and progesterone pellets were introduced as shown in chart 1. At the end of the experiment the uterus measured only 13 × 11 mm., but the endometrium was surprisingly thick, (3–4 mm. in sections). The glands of the functionalis were secretory and contained almost no mitoses (colchicine technic). The low surface epithelium was likewise almost devoid of mitotic figures.

The hormone pellets introduced into this animal brought the endometrium well into the secretory phase, although the uterus as a whole remained very small.

Monkey 393. This early mature female was castrated on Feb. 1, 1938, day 23 of the cycle. The ovaries were rather small (8.5 × 6 × 6 mm.), but contained corpora albicantia and one current corpus luteum. Bleeding occurred 2 days after castration, uninhibited by the estrone injections (100 R.U. daily). At the end of the experiment (chart 1, series *A*) the uterus measured 17.5 × 15 mm., but the endometrium was only 2.5 mm. in thickness in the fresh state, nearly the same in sections. Epithelial structures were quite comparable to those of progesterone-stimulated animals (chart 1, and fig. 19, 20). The surface epithelium was thin, with

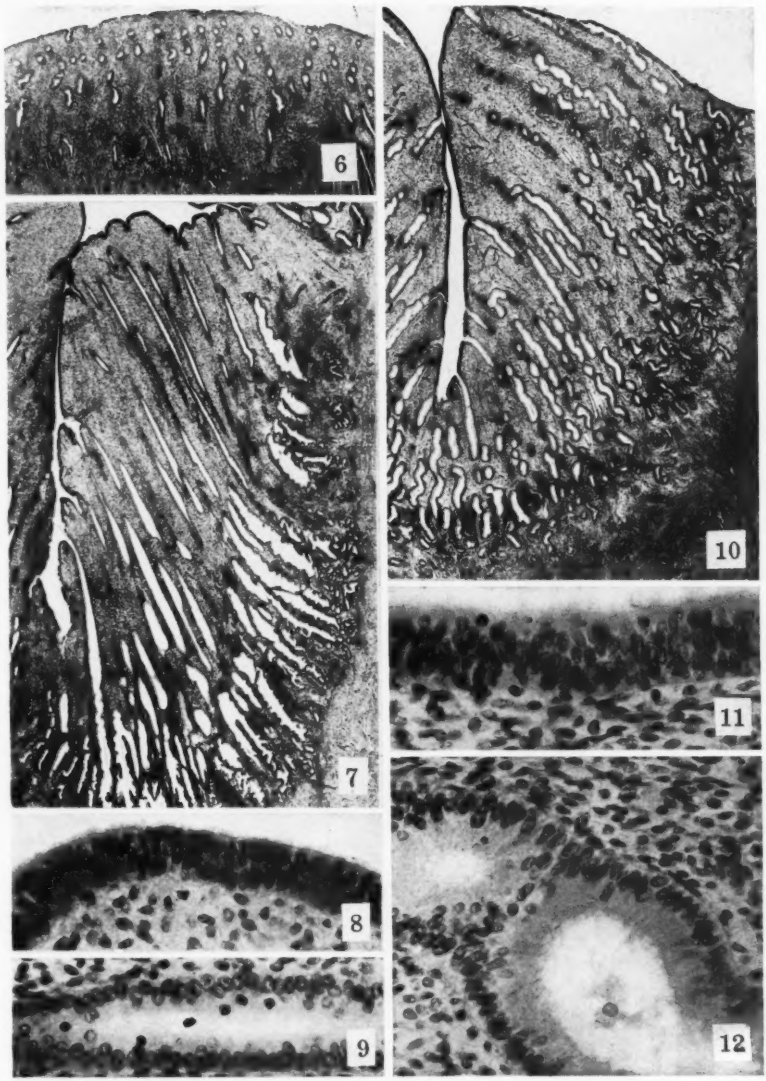

PLATE II., Fig. 6, 7. ENDOMETRIUM OF NO. 454 BEFORE AND 19 DAYS AFTER IMPLANTATION OF PROGESTERONE PELLETS, (both ×15). Fig. 8, 9. SURFACE EPITHELIUM AND UTERINE GLAND FROM FIG. 7 (×300). Fig. 10, 11, 12. ENDOMETRIUM OF NO. 581 (chart 1), LOW POWER (×15); SURFACE EPITHELIUM AND GLAND (×300); PROGESTERONE AND TESTOSTERONE PELLETS FOR 13 DAYS.

nuclei nearly filling the cells. The nuclei of the gland epithelium were basal and arranged in a single layer. There was no evidence of secretion.

This female menstruated 2 days after castration, unhindered by the injection of 100 R.U. per day of amniotin (chart 1). With estrin-testosterone treatment the epi-

thelial structures of the moderately thickened endometrium approached the normal condition of progestin treated organs but with no evidence of secretion.

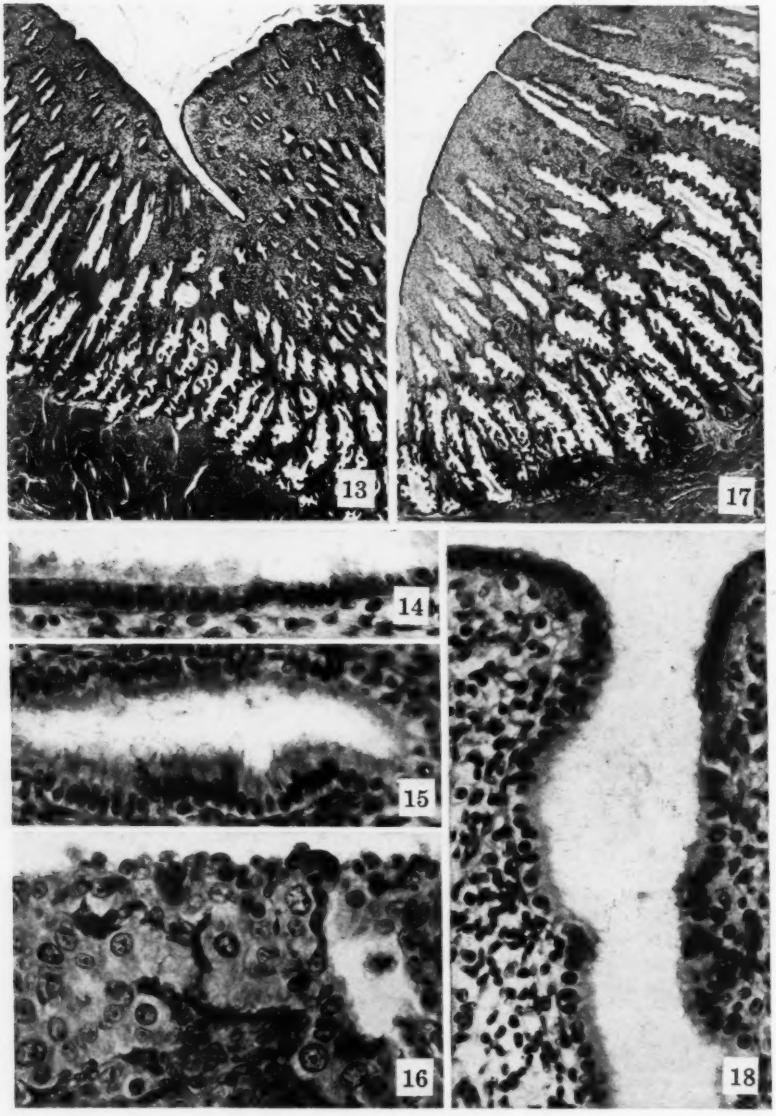

PLATE III., Fig. 13. ENDOMETRIUM OF NO. 543 (chart 1), PROGESTERONE BY INJECTION. Fig. 14, 15. SURFACE AND GLAND EPITHELIUM FROM FIG. 13 (×300). Fig. 16. SURFACE LAYER OF ENDOMETRIUM OF NO. 539 (chart 1) SHOWING 'PLACENTAL' REACTION DESPITE TESTOSTERONE INJECTION ALONG WITH PROGESTERONE (×300). Fig. 17. ENDOMETRIUM OF NO. 540 (chart 1), TESTOSTERONE AND PROGESTERONE BY INJECTION FOR 19 DAYS. Fig. 18. DETAIL ×300 OF FIG. 17.

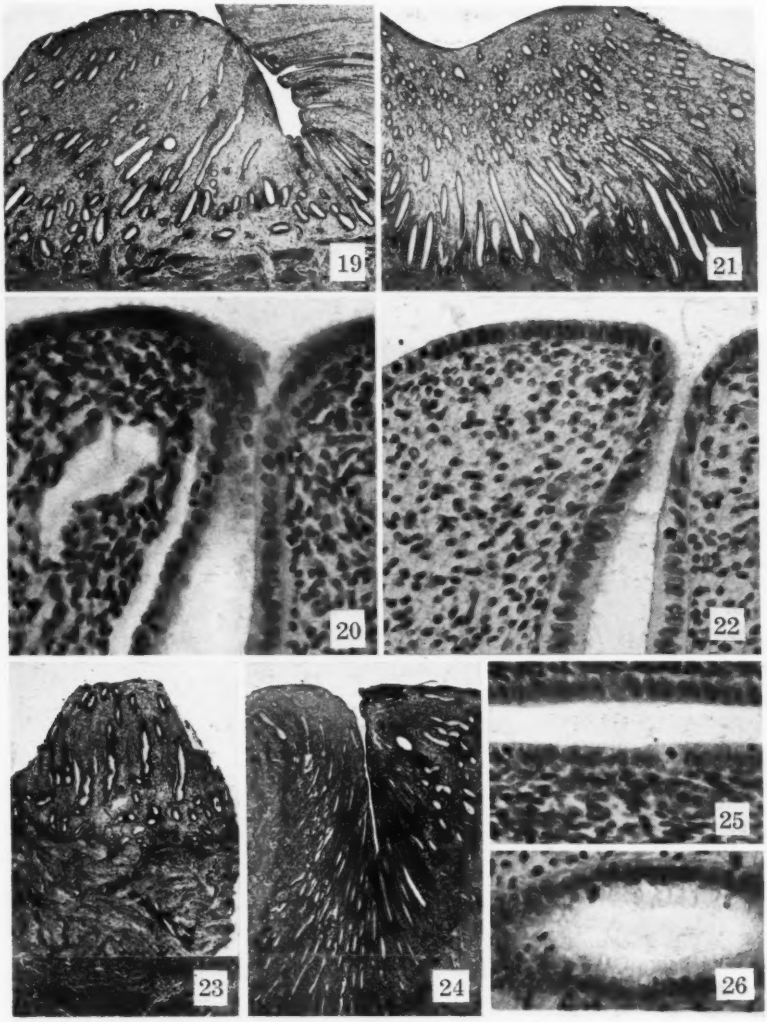

PLATE IV., Fig. 19, 20. ENDOMETRIUM OF NO. 393 (chart 1), ×15 AND ×300; TESTOSTERONE FOR 19 DAYS BY INJECTION. Fig. 21, 22. ENDOMETRIUM OF NO. 602 RECEIVING 50 mg./d. TESTOSTERONE PROPIONATE FOR 21 DAYS (×15 and 300). Fig. 23, 24. ENDOMETRIUM OF NO. 256 BEFORE AND AFTER TESTOSTERONE TREATMENT (×15). Fig. 25, 26. SURFACE AND GLAND EPITHELIUM FROM FIG. 23 (×300).

Monkey 541. When received on April 22, 1937, this animal weighed 3620 gm., 3980 gm. when the experiment began. The autumn cycles were 20, 25 and 34 days in length, all non-ovulatory. In October and November the sex skin was swollen in the mid-line of the perineum. In December the swellings retrogressed completely, nor were they revived during the subsequent hormone injections (chart 1, series A). The small ovaries (7.5 ×6 ×4.5 mm.) were removed on Feb. 1, 1938, day 28 of cycle. A uterine biopsy was taken on Feb. 24, 10 days after injection of testosterone was begun. The biopsied mucosa differed in no way from

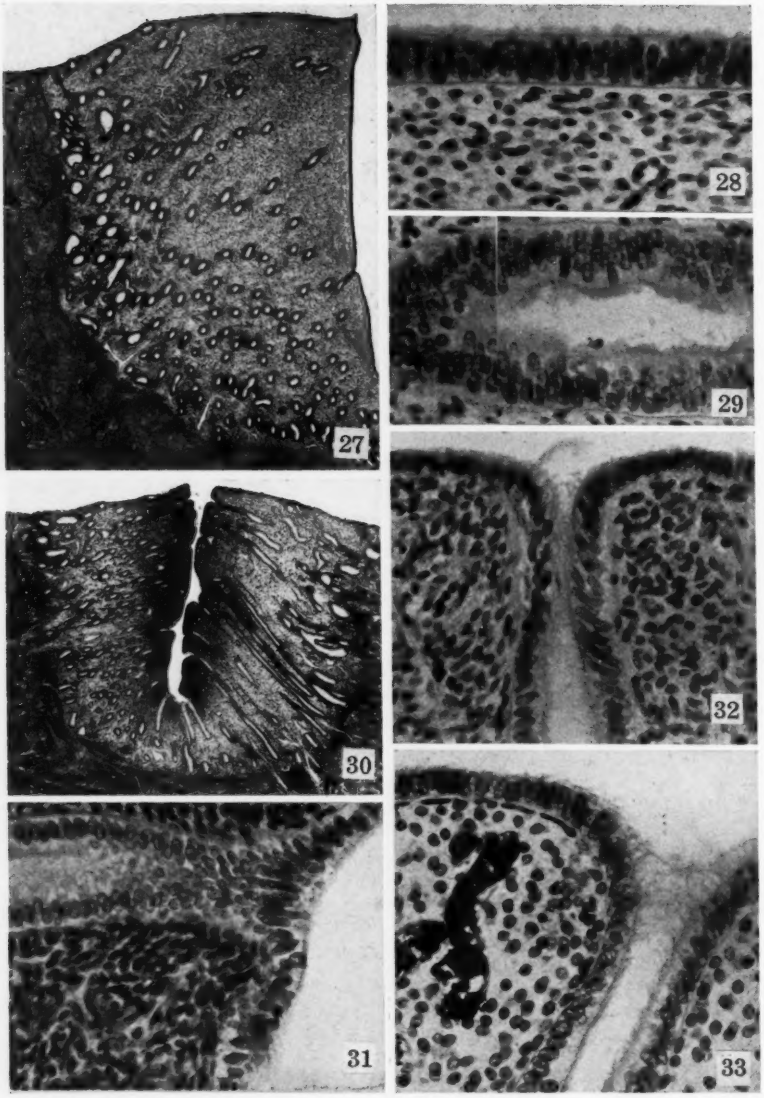

PLATE V., Fig. 27, 28, 29. MUCOSA (×15) AND SURFACE EPITHELIUM AND GLAND (×300) OF NO. 569 (chart 1); TESTOSTERONE PELLETS FOR 13 DAYS. Fig. 30, 31. ENDOMETRIUM OF NO. 330 (×15 and 300), EXPOSED TO TESTOSTERONE WITHOUT ESTROGEN FOR 63 DAYS. Fig. 32. ENDOMETRIUM OF NO. 591 (×300), RECEIVING 750 mg. TESTOSTERONE PROPIONATE IN 17 DAYS. Fig. 33. THAT OF NO. 620 RECEIVING 270 mg. IN 20 d.; BOTH THIS AND THAT OF FIG. 32 ARE NON-CASTRATES.

that removed at autopsy 8 days later. With progesterone such an added exposure to the hormone would have resulted in much further differentiation (cf. 539). In both specimens the epithelial structures were low as in No. 393 described above. The gland epithelium of 541,

however, differed in the exaggeration of the light basal zone described by other workers but absent from most of the preparations of the present paper.

This previously non-ovulating monkey furnished an endometrium practically the same as that of No. 393, a previously ovulating female treated with estrone and testosterone in an identical fashion.

Monkey 565. This 4640 gm. female menstruated Sept. 28 and Nov. 13, 1937, these dates marking a 47-day non-ovulatory cycle. On Dec. 6, a 12-day embryo was removed by hysterotomy. Menses occurred Jan. 10 and Feb. 3; this cycle was non-ovulatory. Castration was performed on Feb. 16, 1938, day 14 of the cycle. One ovary measured 10 ×6.5 ×5.8 mm. and contained a 3 mm. follicle; the other ovary measured 9 ×5 ×5 mm., the uterus at autopsy 16 ×12.5 mm., the fresh endometrium 2.5 mm. in thickness and a little less in sections. The effect of the hormones (chart 1, series *B*) was mostly estrogenic, epithelial structures for the most part being moderately thick with pseudo-stratified nuclei and many mitoses brought out by the colchicine technic.

The estrogenic effect greatly predominated in this case over anything that might suggest response to progesterone. There was no indication of secretion.

Monkey 569. The autumn 1938 cycles of this 4240 gm. female were 53, 25, 27 and 23 days in length, all non-ovulatory. Sex swellings appeared in September, not again afterward, not even during the course of injection (chart 1, series *B*). The medium-sized 'empty' ovaries (9 ×6 ×6 mm.) were removed Feb. 16, day 12 of cycle. At autopsy the uterus measured 17.6 ×14.5 mm., the endometrium fully 2 mm. in thickness in the fresh state, almost the same in sections. The epithelial structures were fairly thick and pseudostratified, as obtains near the middle of normal menstrual cycles and up to the 8th day after ovulation. Progesterone-like effect was absent. Colchicine 'fixed' an enormous number of mitotic figures throughout the mucosa.

Testosterone pellets (chart 1) in conjunction with estrin failed to bring the mucosa beyond an advanced proliferative stage such as is found around the time of ovulation in the normal cycle.

Monkey 539. This female was received April 22, 1937, weighing 4660 gm. By Feb. 1, 1938, day 27 of the cycle, when she was castrated, she weighed only 4030 gm. the loss in weight being due to tuberculosis; yet she responded to the injections of hormones (series A, chart 1). Cycles of 33, 20 and 20 days were non-ovulatory, the uterus remaining under 14 mm. in width. A uterine biopsy made on Feb. 24, 1938, showed the uterus to be in a state of early secretion. Eight days later this condition had greatly advanced. A comparison of the surface epithelium at the time of biopsy with that removed at autopsy demonstrates its progressive thinning. The gland epithelium had also thinned somewhat in the interval. In figure 16 may be seen the 'placental reaction' developed in response to surgical trauma on the progesterone-sensitized uterine epithelium (23, 25).

The advancement of the secretory phase of the endometrium within an 8-day interval may be followed in this case. Testosterone failed to inhibit (or to enhance) the effect of progesterone or to prevent the development of the 'placental reaction' of the uterine epithelium.

Monkey 540. On arrival on April 22, 1938, this animal weighed 4060 gm.; 3 months later, 4330 gm. The autumn cycles of 36, 35, 27 days were all non-ovulatory. It follows, therefore, that the endometrium of the animal had not been in the pregravid phase for practically a year. When castrated on Feb. 1, 1938, day 21 of the cycle, the ovaries were small (9 ×6 ×4 mm.). Exposure to all 3 hormones (chart 1, series A) for 19 days resulted in a much enlarged uterus (18 ×16.5 mm.) with very thick well developed endometrium. The advancement into the luteal phase is clearly expressed in the structure of both glands and surface epithelium (fig. 17, 18).

The endometrium of this animal receiving testosterone in addition to progesterone

compares favorably with that of *monkey* 543 receiving no testosterone. There is no clear indication of either synergism of testosterone with or antagonism to progesterone with reference to its effect on the endometrium.

Monkey 579. Weight of this animal was 4650 gm. when received Nov. 16, 1937, 4510 gm. in March 1938. Castrated on Feb. 1, day 10 of the cycle, she received pellets of all 3 hormones implanted according to chart 1 (series *B*). After 13 days the endometrium was 3.5 mm. in thickness in the fresh state, the uterus measured 16×13 mm. The surface epithelium was thinner than that of 581 (fig. 11) treated in the same way or about like that of 454 shown in figure 8. The stage reached in the endometrium in this case corresponds to that about 12 days after ovulation in the normal menstrual cycle.

Hormone pellets in 13 days brought the endometrium into a less advanced stage than that attained by injection of the same three hormones in 19 days in the last two cases listed (*monkeys* 539 and 540).

Monkey. 581. This animal weighed 5240 gm. on Nov. 16, 1937, 4270 gm. when killed on March 9, 1938, the weight lost being due to tuberculosis. When removed on Feb. 16, day 16 of cycle, the ovaries were small (8.0×5.8×5.0 mm.). The animal received pellets of all 3 hormones (series *B*, chart 1). At autopsy, the endometrium measured 5 mm. in thickness in the fresh state; in sections (fig. 10) it appears thicker than that of 579 treated in the same way. The glands were in the secretory phase (fig. 12) corresponding to about 10 days after ovulation in the normal cycle; the surface epithelium was transitional (fig. 11).

In this case thirteen days exposure to all 3 of the steroidal hormones again brought about changes equivalent to estrin and progestin alone, i.e., without testosterone (cf. No. 544 and 452).

It is apparent from the foregoing protocols that in the castrated monkey testosterone, in the doses and combinations used, had little effect on the endometrium. Certainly in no case was the secretory phase even approximately attained with testosterone alone or in conjunction with estrone. In one regard only does testosterone seem to simulate the progesterone effect, namely in the thinning of the surface epithelium as compared with the estrous condition. A cooperative or synergistic effect, that is, an enhancement of proliferation induced by estrone or of progestational changes brought about by progesterone, is vaguely sugested but by no means demonstrated. With the exception of modifying the height of cells and nuclear arrangement of the uterine epithelium testosterone preserved, for the duration of the injections, the condition of the endometrium obtaining when exposure to the hormone began. In this series no observations were made which could be interpreted as an inhibitory influence on the endometrium.

Further experiments with castrates. (*a*) *Increase in dosage.*

Monkey 492. This monkey, weighing 4050 gm., was castrated on Jan. 6, 1938. In the small ovaries removed there was no sign of previous ovulation. From Jan. 7 to 12 inclusive she received 50 R.U. of amniotin daily, from Jan. 13 to 22 inclusive 25 R. U. of amniotin and 25 mg. daily of testosterone propionate. No bleeding had occurred by the time the animal was killed on Jan. 24, 10 hours after administration of colchicine. During the injections the intensity of color and the swellings of the sex skin increased but the amount of vaginal desquamation remained at 5% (cf. chart 1). In sections the fairly dense, nonsecretory endometrium measured about 3 mm. in thickness and showed numerous mitoses. The glands were somewhat dilated at the base and their walls were made up of the pseudostratified epithelium characteristic of the estrous picture. The surface epithelium, however, was low. Unlike progesterone, the testosterone enhanced rather than counteracted the effect of estrin on the color and the swellings of the sex skin.

Monkey 602, a 4040 gm. female, was received on March 3, 1938. Cycles after Sept. 15

were 25, 30, 27, 21 days in length. She was castrated on March 2, 1939, and the ovaries found to be 'empty.' After priming with 100 R.U. of amniotin daily, from March 17 to 29, 25 R.U. of amniotin plus 50 mg. testosterone was injected daily (except on 2 Sundays) for 17 days (total, 0.750 gm. testosterone!). Colchicine was injected at 10:00 A.M. April 20, 5 days after last injection, and the animal was killed at 4:30 P.M.

The mucosa was of medium thickness, the surface epithelium low, with roundish single-ranked nuclei, still, however, showing numerous mitoses; the basal glands show more marked estrogenic effect than those of the functionalis, whose nuclei were in single rows with basal clear zone (glycogen?) below them in some cases.

Raising the dosage to 25 or even to 50 mg. daily produced in 2 animals endometria which differed in no essential regard from those produced by 10 mg. daily. There resulted neither a further approach to a progestational condition nor recognizable increase in proliferative (estrogenic) activity.

(*b*) *Biopsy of the endometrium before and after the daily administration of 25 mg. of testosterone acetate* was made in the case of No. 256. The protocol of this case is as follows.

Monkey 256 had been variously used during the 5 years she was a member of the colony. Her menstrual intervals seldom varied from the 24 to 28-day rule. From Jan. 3, 1938, day 5 of the cycle, she received 100 R.U. of amniotin daily and on the last day the one surviving ovary was removed and a uterine biopsy made. This showed a typical, mildly estrous condition, with fairly thick, pseudostratified surface epithelium. The gland epithelium likewise showed estrin influence in the high cells with staggered arrangement of the nuclei. Immediately after castration and biopsy, 50 R.U. amniotin plus 25 mg. testosterone acetate was injected daily for 10 days. A slight bleeding occurred on Jan. 18 and 19 only (i.e., during the course of injections), the 9th and 10th days after castration. At autopsy on Jan. 24 (colchicine technic) the endometrium was no thicker than at biopsy (1.1 to 1.2 mm.) and less succulent; the surface epithelium was very thin (fig. 24). The gland epithelium had also changed to a lower phase, with roundish basal nuclei. The specimen at autopsy despite the use of colchicine showed much lower mitotic activity than the untreated sample removed 10 days previously.

Biopsy of the uterus taken after 7 days administration of estrin was compared with the uterus removed after subsequent administration for 10 days of estrin and testosterone. Mitoses were much less numerous at the end of the treatment and the uterine glands thinner walled. The only changes noted from the last treatment lay in the reduction of the mitotic index and in a lowering of the epithelial structures. The larger dosage probably hastened the usual changes in this direction.

(*c*) *Testosterone without concomitant injection of estrogen.* In view of a growing amount of data bearing on the antagonistic action of estrogen toward testosterone as well as progesterone (6, 15, 25–28), it seemed possible that in the preceding experiments the presence of an estrogen along with the testosterone exercised an inhibitory action on the latter. Three females, therefore, received androgen alone, after being primed with amniotin.

Monkey 463, a 5540 gm. female, furnished a 13-day ovum on Oct. 13, 1936; a 15-day ovum on Dec. 8. She was castrated on the latter date and used in several experiments thereafter. April 22 to May 3, 1937, a 12-day bleeding occurred, induced by injection of amniotin. May 3 to 7, 100 R.U. of amniotin daily was administered, followed by 10 mg. daily of testosterone for 13 days. She was killed 2 days after the last injection.

The endometrium was 1.5 mm. in thickness in sections, the surface epithelium of minimal thickness with rounded nuclei. In the gland epithelium the nuclei were basal although some glands showed a light basal zone indicative of glycogen storage, a condition seldom seen in

the course of these experiments. A few mitotic figures could be found in the glands, more in the uterine epithelium. There was no secretory activity whatever.

Monkey 305. This mature monkey gave birth on Dec. 18, 1936, but was not allowed to lactate. She menstruated Feb. 11, March 6 and March 30, 1937. The large, infected surviving ovary was removed on April 14, from which date, until autopsy on April 24, she received 10 mg. daily of testosterone acetate. The endometrium almost exactly duplicated that of 463.

Monkey 585 (4350 gm.) was hysterotomized and had one ovary removed on Nov. 3, 1938. On March 20, 1939 she furnished an ovary containing a ripe follicle with ovum containing second maturation spindle. She was primed April 3 to 12 with 50 R.U. per day of amniotin; April 13 to 30 inclusive she received 10 mg. per day of testosterone propionate. She was killed 4 days after the last injection. The uterine mucosa in this case proved similar to the foregoing, was thin, mildly proliferative, with mitoses easy to find in the glands, but sparse in the surface epithelium, which was characteristically low.

The foregoing 3 cases which received only testosterone (propionate or acetate) furnished uteri which differed little from those subjected to both estrin and androgen at the same time. They are listed among the least stimulated uteri of the whole experimental series here reported and demonstrate the comparative inadequacy of testosterone as a stimulus to cell division in the endometrium.

(d) *Testosterone alone for 63 days. Monkey* 330 was given 10 mg. of testosterone acetate daily for 60 days and was killed, after pre-treatment with colchicine, 3 days after the last injection (29). The following protocol details the findings.

Monkey 330. Since her arrival in the colony Nov. 20, 1934, weighing 4810 gm., (later up to 5680 gm.), this female was used in various ways. On Nov. 19, 1936, she furnished a 12-day embryo, the very next month, a 11-day embryo. The right ovary was removed at the first hysterotomy, the left at the second. After 5 days priming with 100 R.U. of amniotin daily (April 23 to 27, 1937) the animal received 10 mg. of testosterone acetate daily for 60 days, was killed on the 63d day. The uterus was normal in size, the endometrium 1.5 to 2.0 mm. in thickness in the fresh state about the same in sections (fig. 30). The surface epithelium was low (fig. 31), the nuclei broadly elliptical, in the low simple gland epithelium the nuclei tended to be roundish.

After 60 days treatment with testosterone alone the endometrium was in a good state of preservation and far from atrophied. The epithelial structures were thin but not in the 'resting' state or that produced by moderate estrin administration. There was, of course, no evidence of secretion in the glands.

Experiments with Non-castrates

Because of the now well-established reciprocal relations between gonads and the anterior pituitary and the effects of fat-soluble sex hormones on the latter (31, 32, 33), it seemed necessary to repeat some of the foregoing experiments on normal, non-castrated monkeys with both low and high dosages of testosterone. The following 4 pertinent experiments are cited.

Monkey 620. This 4860 gm. female was received on March 4, 1938. Cycles (after Sept. 19) were 46, 23, 35, 23, 36 and 28 days in length, several of them ovulatory but not the last one. Beginning on April 14, day 16 of the cycle, 15 mg. of testosterone propionate was injected daily (except on 3 Sundays) until May 10. Bleeding began on June 2 after a latent period of 20 days. But on May 4, 20 days after injections began, a biopsy of the uterus was made. At this time the endometrium was only mildly proliferative, or about as one would expect to have been the condition when the injections began. The presence of the ovaries in no way affected the result as compared with castrated cases.

Monkey 670, a 3510 gm. female, received Feb. 28, 1939, proved to be amenorrheic. From April 21 to May 8 (with the exception of 3 Sundays) she received 25 mg. of testosterone

propionate, a total of 375 mg. over an 18-day period. After killing the animal with fuel gas the blood vessels were filled, via the abdominal aorta, with India ink. The uterus was not much enlarged over that accompanying small ovaries containing follicles up to 0.75 mm. in diameter. A high degree of vascularity was apparent in sections of the uterus. Proliferation was not marked, the endometrium remaining thin. In 10 sections 4 mitotic figures were counted in the glands, one in the uterine epithelium, which was thin, with round nuclei, as is characteristic of testosterone and progesterone treated specimens. There was little if any indication of secretion in the glands; the chief effect was estrogenic. This action was very evident in the brilliance of the sex skin and the characteristic edematous swellings in the mid-line of the perineum.

Monkey 434. This adolescent female, weighing about 4 kg. had been used in metabolism studies. Her weight in January, 1938 was 4100 gm.; last menstrual period was Dec. 22, 1938. At laparotomy on Jan. 5, 1939 the uterus measured 13 mm. in width, the ovaries were small, hence the cycle non-ovulatory; the ovaries were not removed. From Jan. 7 to 13 inclusive 50 R.U. of amniotin and 25 mg. of testosterone acetate daily, were administered; from Jan. 14 to 22 inclusive the amniotin was reduced to 25 R.U. but the androgen was continued at 25 mg. On Jan. 24, at 9:00 A.M., 0.5 mg. per kg. colchicine was given by stomach tube; at 3:30 P.M. the animal was seen to vomit. She was killed at 7:30 P.M. The uterus had enlarged somewhat, the endometrium was 3 mm. thick in the fresh state, about the same in sections, succulent, full of mitoses. The muscularis was poorly developed. The epithelial structures appeared to be identical with those of No. 393 receiving more estrogen (double), less androgen (40%) and for 3 days less time. In a similar stage, in the progesterone treated organ, mitosis would have slowed down greatly or ceased altogether.

Monkey 591. This 4280 gm. female was received Feb. 2, 1938. Ovulatory cycles were recorded in the autumn; on Jan. 18, 1939 hysterotomy was performed and one ovary removed; second hysterotomy was made on March 12. The menses reappeared on April 11 and beginning April 20, 50 mg. testosterone propionate was injected daily (except on 2 Sundays) until May 6 (750 mg. over a period of 17 days). Biopsy of uterus was made on the 4th day after the last injection. The biopsy showed that proliferation of the endometrium had proceeded no further than in castrates receiving but 10 mg. testosterone, as reported above. Mitoses had almost completely ceased, indicating that with the dosage used, the 'estrogenic' activity of the androgen was coming to an end. The uterine epithelium was low. The stroma was fairly compact. There was no evidence of secretion.

In the experiments just cited, 4 favorable animals were treated for 20, 18, 18 and 17 days with 15, 25, 25 and 50 mg. respectively of testosterone propionate daily. The results were much the same as already described for castrates. The presence of ovarian tissue proved ineffective in modifying the results, nor did the huge dose in the case of the last subject (591) enhance the 'testosterone' effects. Its estrogenic value indeed was less than in smaller doses and its 'progesterogenic' effect negligible.

Testosterone Administration During an Ovulatory Cycle

Monkey 576. This 5330 gm. female menstruated on Jan. 3, 1938, for the first time after her arrival in Oct., 1937. Palpation disclosed ovulation Jan. 15 to 16, a diagnosis corroborated by laparotomy on Jan. 21. From this date on for 7 days 25 mg. of testosterone propionate per day was injected, a total of 175 mg. Colchicine was given at 12:01 A.M. on Jan. 29 and at 10:00 A.M. colloidal HgS was injected intravenously just before killing. The uterus was frozen in situ by the Altmann-Gersh method. The endometrium measured up to 5 mm. in thickness after fixation (fig. 5) The glands were in an advanced stage of secretion, mitoses were all but absent from both gland and surface epithelium. The latter was as thin as in normally the late pregravid phase.

Twenty-five mg. daily of testosterone propionate injected from day 19 of the menstrual cycle or the fifth day after ovulation for 7 days (total dosage 175 mg.) had no recognizable effect on the development of pregravid changes in the endometrium, for these manifested themselves as in the normal cycle.

DISCUSSION OF DATA BEARING ON THE EFFECTS OF TESTOSTERONE ON THE MONKEY UTERUS

The experimental animals. It may be seen from a perusal of the 35 protocols presented above that most of the subjects of the experiments were choice animals, indeed among the best in the Carnegie colony. Many of the females, before being used for these experiments, had yielded one or more embryos; such breeding activity is the best proof of the adequacy of the subjects' own hormones, for ovulation, conception and nidation of the ovum is as much as the student of reproduction can ask of a wild animal in captivity. Some of the animals, it is true, had tuberculosis, in fact were used because they had to be destroyed anyway. That this disease interferes only in the advanced stages with reproduction, however, is true of the monkey as of the human female.

The importance of selecting animals of good quality for experiments is not sufficiently often emphasized, for the physical condition of the subject has much to do with its response to hormones. It is a mistake to expect an introduced hormone to make up for the subject's general unsoundness. Even though one's attention may for the moment be focused on a specific effect, the interrelation of all the endocrine glands as well as other organs is such that it is essential that the entire organism be in as nearly prime condition as possible.

The affected organ (*Erfolgsorgan*). It is becoming more and more apparent that, to judge the action of a sex hormone—whether androgenic or estrogenic, synergistic with or antagonistic toward other hormones—it is necessary to investigate each substratum or affected organ individually, as has recently been well stated by Mühlbock (33). Different tissues of a given organ, in fact, must be considered separately, e.g., stroma, muscle and epithelial structures of the uterus. Thus, even among the last mentioned, surface epithelium and superficial and basal portions of the glands show differences, at least in rate of reaction to hormone stimulation.

The bisexual nature of the hormones. Since Butenandt and Kudzus (34) first established the estrogenic action of testosterone in the infantile female mouse further experimental data, particularly through the work of Korenchevsky and associates (35–38), render it likely that all of the steroidal hormones will be found to be bisexual in nature. Experiments with low doses seemed, however, to point to progesterone as an exception, i.e., as a purely female hormone (38). However, Lamar (39) has shown that administered in sufficient quantity (5 mg. per day) progesterone restores and maintains the accessary organs of the castrated male rat.

Estrogenic action of testosterone on the monkey endometrium. Proliferation of derivatives of the mullerian duct and of the mammary glands and the maintenance of other secondary sex characters of the female organism constitute evidence of estro genic action. A positive test implies increase in weight of the organ due, first, to increased edema of the tissues and, second, to cell multiplication, The presence of mitoses, made more manifest by the colchicine technic, is further indication of proliferation.

In this paper an estrogenic action of testosterone on the endometrium can hardly be said to be demonstrated, but only the ability of the androgen to maintain the endometrium in about the status attained when the administration of the androgen began (cf. 40, 41). Castrate atrophy was in no case corrected but only prevented by testosterone. This distinction was pointed out by Engle and Smith (42), who found that, while testosterone was incapable of producing a pregravid endometrium in the monkey, it did for a time prevent the involution of the pregravid organ previously built up by the usual estrin-progestin procedure.

The foregoing protocols further amply demonstrate the fact that testosterone does not antagonize the action of estrogens in the endometrium. On the other hand, no clear case of synergistic action was encountered. There was a tendency of the uterine glands to become dilated basally, as in estrogen-treated organs and, more especially, in those treated by estrogens to which a small amount of progesterone has been added (43).

No giant uteri, such as Korenchevsky and Hall (37) found in the rat, were seen; in fact, no undue stimulation of the muscularis was observed in any of the experiments, not even when as much as 50 mg. of testosterone propionate was administered daily.

On the sex skin, however, estrogenic action of testosterone was noted without exception, for throughout the experiments with this hormone the sex skin was always brilliantly red (22, 29, 43). Progesterone is antagonistic to estrin in this regard (24); even in pregnancy the writer was able to blanch the sex skin with a sufficiently high dosage of progesterone. In the present series of experiments, however, the concurrently injected estrogens and testosterone or both were sufficient to override the blanching action of the progesterone.

Testosterone and progesterone compared. As testosterone failed to conteract the action of estrogens (cf. 44) so it also failed to interfere with the action of progesterone on the endometrium. Not only did the full progestational changes unfold themselves in the presence of both progesterone and testosterone (*monkey 540*, for example) but in the case of No. 539 the placental reaction of the surface epithelium manifested itself. Hisaw (23) showed this could be elicited in the monkey in the progesterone-sensitized uterus under the stimulus of trauma (cf. also 25). Courrier and Gros (28) likewise saw placentomata develop in the rat receiving injections of testosterone. With testosterone alone, however, in hypophysectomized rats or x-ray sterilized mice Parkes and Zuckerman (45) failed to get any luteinization or decidual reaction.

Contrary to the findings in the rabbit (17, 18, 19) testosterone completely failed to bring about progestational conditions (serration of the gland surface and secretion) in the monkey uterus when used alone. In only one regard did the testosterone-treated mucosæ tend to approach those treated with progesterone, namely in the progessive transformation of the epithelial structures, particularly the surface epithelium. In the presence of estrogens alone, as, for example, in the middle of the menstrual cycle, the surface epithelium was tall, columnar, with pseudostratified nuclei arranged in two to three levels. Upon sufficient exposure to testosterone the cells become cuboidal, the nuclei broadly elliptical. The glands in the functionalis showed such changes more slowly, the basal ends of the glands not at all, within the time limits of the present experiments. These changes were similar though perhaps more marked with progesterone treatment, as the illustrations show, than in the normal cycle. These results are at variance with those of Phelps, Ellison and Burch (41) who noted a well-marked pseudostratified condition in the surface epithelium, mild pseudostratification of the uterine glands in testosterone treated, castrated guinea pigs.

The low epithelium produced by progesterone or testosterone differs from the atrophic condition. In the latter the cells, though low, are slender and the nuclei closely packed and dense; the progestational epithelium has broad cells with more vesicular nuclei. In all of the experiments the subjects were first primed with estrogen, a procedure which previous experience indicated resulted in a proliferating mucosa. This was corroborated by appropriate biopsy in *monkey 256*. It would be interesting, however, to study monkey uteri awakened by testosterone after profound castration atrophy.

Castrated vs. normal females. Both in the presence and in the absence of the ovaries testosterone failed to bring about the classic progestational changes which in minimal doses may readily be produced with progesterone, either in normal or in castrated monkeys. With testosterone the results were identical in the normal and the castrate at all dosage levels from 10 to 50 per day and in periods of time varying from 13 to 19, in one case to 63 days.

SUMMARY OF PART II

Eighteen experiments were performed to discover the effect of testosterone, either injected alone or concurrently with an estrogen or progesterone or both on the monkey endometrium. The animals were for the most part acclimated and many had already demonstrated, by ovulating and conceiving, the efficiency of their own endocrines. Most of the subjects were castrated, usually at the beginning of the experiment; but a few non-castrates were also used. The dosage employed varied from 10 to 50 mg. per day and the time from 13 to 19 days; in one case injections were continued for 60 days.

A progestational endometrium, with serrated secretory glands, was never attained with testosterone alone or in combination with estrogens. A small imitation of progestin action was observed in the migration of nuclei in the surface epithelium and to a lesser degree and later in point of time in the superficial portion of the glands. Unlike the rabbit uterus, therefore, that of the monkey failed in these experiments to respond to testosterone in the characteristic manner in which it responds, in the castrate or normal monkey, to a hundredfold lower dosage of progesterone.

On the other hand, no evidence was found for any antagonistic action of testosterone either to estrogen or to progesterone when concomitantly injected with one or both of these ovarian hormones. The action of testosterone on the sex skin is, however distinctly estrogenic, i.e. antagonistic to progesterone.

The setup of the present experiments does not warrant the statement that testosterone has estrogenic action on the monkey uterus since in no case had castrate atrophy set in when the injections began. It is evident, however, that testosterone is able to maintain an endometrium already built up by estrogen and Engle and Smith's generalization, applied to the pregravid uterus, is extended to the proliferative endometrium as well.

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THE WORK PERFORMANCE OF ADRENALECTOMIZED RATS TREATED WITH CORTICOSTERONE AND CHEMICALLY RELATED COMPOUNDS

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THE NORMAL rat is capable of continued work of the gastrocnemius muscle for several days. Following removal of the adrenal glands the capacity for sustained work is lost within a few hours but this can be prevented by the administration of cortin. In the present investigation corticosterone and compounds which are related to it in structure have been studied in respect to their effect on the capacity of the adrenalectomized rat to perform work.

METHODS

Male rats of the Sprague-Dawley strain which weighed approximately 180 gm. were used in these experiments. The animals were anesthetized with phenobarbital sodium, and bilateral adrenalectomy was performed. Immediately following the operation one gastrocnemius muscle of each animal was weighted with 100 gm. and stimulated to contract 3 times per second. Each animal received 5 cc. of water by subcutaneous injection at the beginning of the experiment and again 6 hours later. The details of the method have been described (1, 2). In this study the intensity of the faradic stimulation was increased above that used in previous studies. This change had the effect of decreasing the period that the untreated adrenalectomized animals were able to work. The compounds to be tested were dissolved in sesame oil and were injected subcutaneously at the beginning of the experiment and again 6 hours later in those animals which had continued to work for this period. Since some animals ceased working within 6 hours the amount of the compound given at each injection (and not total dosage) is used in the summary of results.

RESULTS

As controls 25 rats were bilaterally adrenalectomized and made to work without treatment until collapse. Twelve normal rats, subjected to false operations, were made to work without treatment for 24 hours. The adrenalectomized animals showed a complete loss of muscular responsiveness within 4 to 20 hours after beginning work, whereas the normal animals were all working vigorously at the end of the 24-hour period. The total amounts of work performed by the animals of these 2 groups are represented as controls in figures 1, 2 and 3. The work performance of rats possessing their adrenal glands is clearly differentiated from that of untreated adrenalectomized rats within the 24-hour test period.

In experiment 1, (fig. 1) a comparison was made of corticosterone and desoxycorticosterone acetate. The dilution of these compounds in sesame oil was 1 mg. per cc. when amounts up to 1 mg. per dose were injected and 2 mg. per cc. when amounts

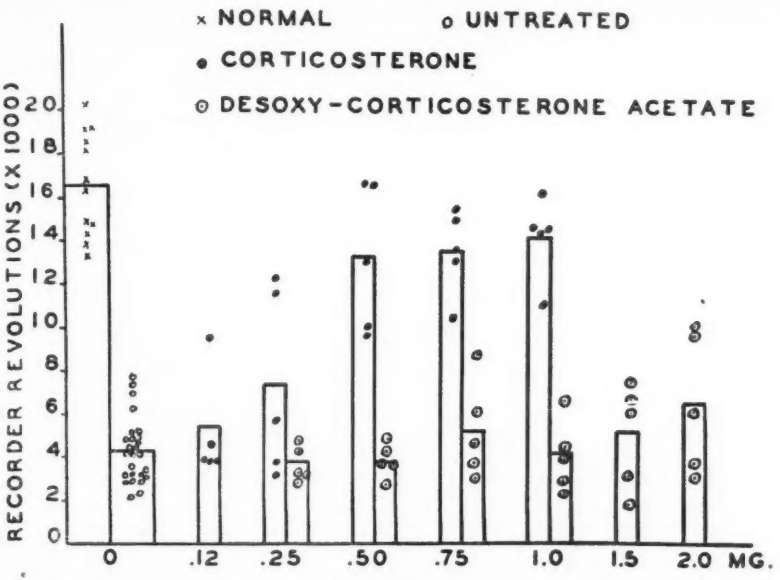

Fig. 1. Group averages and individual records of work performance. Each recorder revolution is equal to approximately 400 gm.-cm. of work. The duration of work is discussed in the text.

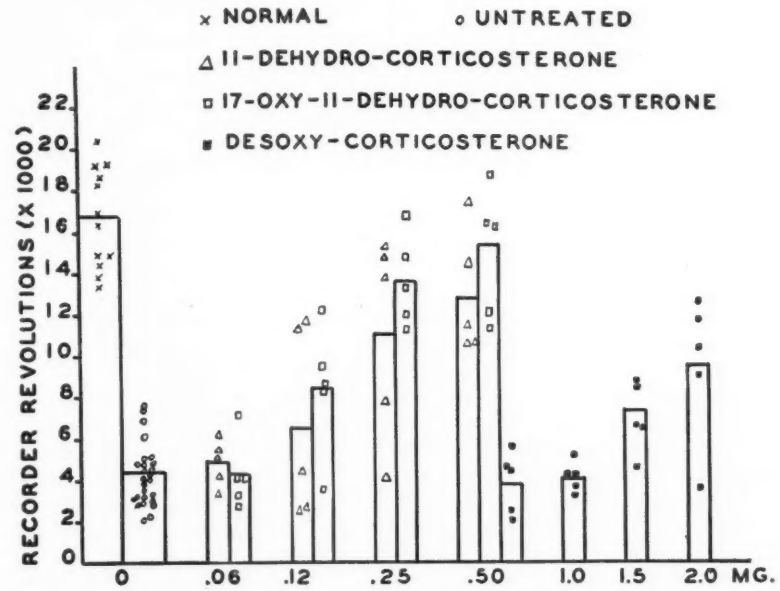

Fig. 2. Group averages and individual records of work performance. Each recorder revolution is equal to approximately 400 gm.-cm. of work. The duration of work is discussed in the text.

over 1 mg. per dose were injected. In the initial comparsion, doses of 0.5 mg. were injected. This amount of corticosterone allowed the adrenalectomized animals to work in a nearly normal manner but no effect of the desoxy-corticosterone acetate was observed. Both smaller and larger doses of each compound were tested. The work performed by those rats which received the corticosterone was clearly greater than the work performed by the animals which received the desoxy-corticosterone acetate.

In experiment 2, (fig. 2) a comparison was made of 11-dehydro-corticosterone, 17-oxy-11-dehydro-corticosterone, and a sample of desoxy-corticosterone which had been saponified from its acetate. The dilutions of these compounds in sesame oil were

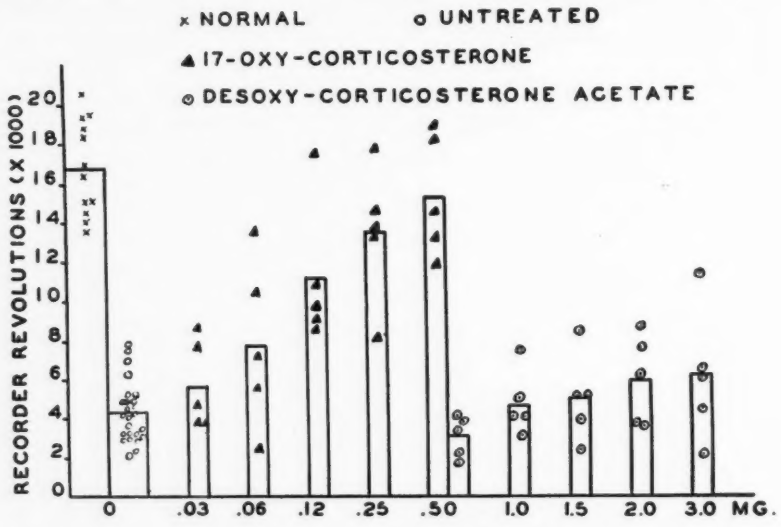

Fig. 3. GROUP AVERAGES AND INDIVIDUAL RECORDS OF WORK PERFORMANCE. Each recorder revolution is equal to approximately 400 gm.-cm. of work. The duration of work is discussed in the text.

identical with those of experiment 1. In the initial comparison 0.5 mg. of each compound was injected. The work performance of those animals which received 11-dehydro-corticosterone and of those which received 17-oxy-11-dehydro-corticosterone was definitely increased over that of untreated animals. No effect on work performance was noted from this dose of desoxy-corticosterone. When the size of the dose was increased to 1.5 mg. and 2.0 mg. the work performance was increased. Amounts of 0.125 mg. per dose of both 11-dehydro-corticosterone and 17-oxy-11-dehydro-corticosterone were effective in some instances.

In experiment 3, (fig. 3) a comparison was made of 17-oxy-corticosterone and desoxy-corticosterone-acetate. The dilutions of these compounds in sesame oil were identical with those of experiment 1 and 2. Amounts of desoxy-corticosterone acetate up to 3 mg. per dose had little or no effect on the work performance of adrenalectomized rats whereas a clear enhancement of work performance was produced by doses of 0.125 mg. and greater of 17-oxy-corticosterone.

In experiment 4, a sample of 17-oxy-11-dehydro-corticosterone which had had the ethylenic bond reduced (carbons 4–5) was examined. The following doses were tested: 1 and 2 mg., 3 tests each; 5 mg., 5 tests; 10 mg., 2 tests; 20 mg., one test; and

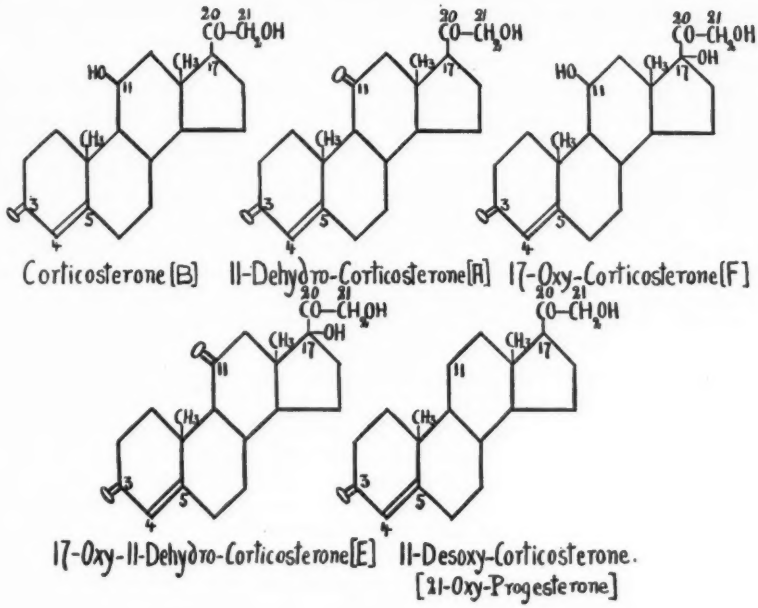

Fig. 4. COMPOUNDS WHICH POSSESS THE PROPERTY OF INCREASING THE WORK CAPACITY OF ADRENALECTOMIZED RATS.

25 mg., 2 tests. The work performance of each of the animals tested came within the range of variability of untreated animals.

In experiment 5, (table 1) a number of substances possessing sex hormone activity were examined for their possible effect of work performance. The following com-

TABLE 1. SUBSTANCES WITHOUT EFFECT UPON WORK PERFORMANCE OF ADRENALECTOMIZED RATS

Substance	Dose tested (mg.)	Rats per dose
Progesterone	2, 5, 10, 20, 45	1
Ethinyl testosterone	1, 2, 5, 10, 20	1
Testosterone	1, 2, 5, 10, 20	2
Testosterone propionate	5, 10	1
Estradiol benzoate	1, 4, 10	2
Stilbestrol	5, 10, 20, 50	2

pounds were examined: progesterone, ethinyl testosterone, testosterone, testosterone propionate, estradiol benzoate and stilbestrol. All of the animals in this experiment worked in a manner characteristic of untreated adrenalectomized animals.

The structural formula of those compounds which had a positive effect on work performance are given in figure 4.

DISCUSSION

The experimental conditions used were selected to produce a severe stress. These sources of stress were the anesthesia, injection of water, starvation and work. The amount of work performed was used as a criterion of normality. This procedure has

been used for several years as a method for rapidly identifying the presence of cortin-like activity in small amounts of test materials. The test was developed because of the theoretical consideration that it would be desirable to require that a substance maintain the resistance of an adrenalectomized animal to a severe stress as adequately as does the adrenal cortical tissue of the normal animal. It has provided a highly specific index as to the presence of those compounds which will completely replace the functional activity of the adrenal cortex.

The relative ineffectiveness of desoxy-corticosterone and its acetate when tested in this experimental situation was surprising since it had been well established that these compounds are highly active in maintaining the life of adrenalectomized animals (3). This discrepancy seems still greater when it is considered that 17-oxy-11-dehydro-corticosterone is relatively inactive (4, 5) as judged by its value in maintaining life, but is highly active as judged by the present results. One possibility is that there may be qualitative differences in the physiologic effects of these compounds. Desoxy-corticosterone and 17-oxy-11-dehydro-corticosterone seem to have the same effects if given in sufficiently large amounts. Therefore if qualitative differences are involved each compound must possess both the 'life-maintenance' property and the 'work' property which are quantitatively dissociated. Such a quantitative dissociation of properties is known to exist among some compounds. For example progesterone is weak in its property of maintaining life in adrenalectomized animals (6) but is high in progestational activity. Desoxy-corticosterone which can be also described as 21-oxy-progesterone is high in life-maintaining activity but is weak in progestational activity (7). It is apparent that slight changes in structure can reverse the order of the quantitative activity of two qualitatively different effects. A second and more simple hypothesis must also be noted. It is probable that at least a part of the discrepancy between the various compounds will be explained as due to differences in the time required for these compounds to be absorbed following injection. Desoxy-corticosterone is very insoluble in water and may be absorbed out of the oil too slowly to be effective in an acute test. The ester of this compound is even more slowly utilized. In addition to the absorption factor there may be differences in the time required for the conversion, destruction and the elimination of these compounds in the body which cause apparent differences in quantitative activity in different methods of assay. The quantitative values for the apparent activity of cortin-like compounds as measured by this test cannot be accepted as indicating the life-maintaining values of these compounds.

The failure of estrogenic and androgenic compounds to benefit the work performance of adrenalectomized rats was expected. The failure of progesterone to do so is significant since Gaunt (6) and his coworkers have proved that it possesses, although only weakly, the property of maintaining life in adrenalectomized rats and ferrets. However, these workers observed that it did not raise the resistance of adrenalectomized rats to water intoxication. Gaunt and his coworkers have pointed out that the failure of progesterone to raise the resistance of adrenalectomized animals to a severe stress may be due either to some qualitative deficiency or to longer time required for utilization.

As judged by the criterion of 'work' the reduction of an ethylenic bond between carbons 4 and 5 of 17-oxy-11-dehydro-corticosterone completely destroys its cortin-like activity.

SUMMARY

The property of maintaining the work capacity of adrenalectomized rats is possessed by corticosterone, desoxy-corticosterone, 17-oxy-11-dehydro-corticosterone,

11-dehydro-corticosterone and 17-oxy-corticosterone. Little or no effect was obtained by the administration of desoxy-corticosterone acetate. A wide discrepancy was noted between the apparent activities of these compounds as judged by the effect on work and as judged by others from the maintenance of life in adrenalectomized animals. This discrepancy may be due to either a qualitative difference in the biologic effects of these compounds or to differences in their rate of utilization. Reduction of the ethylenic bond (carbon 4–5) in 17-oxy-11-dehydro-corticosterone destroyed its effect.

The property of maintaining the work capacity of adrenalectomized rats in this experimental situation was not manifested by progesterone, ethinyl testosterone, testosterone, testosterone propionate, estradiol benzoate, and stilbestrol.

I wish to express my appreciation to Dr. H. L. Mason of the Mayo Clinic who supplied the samples of corticosterone, 11-dehydro-corticosterone, 17-oxy-corticosterone and 17-oxy-11-dehydro-corticosterone in its normal and its reduced forms; Dr. E. Oppenheimer of the Ciba Pharmaceutical Products Co., Summit, New Jersey, who supplied the desoxy-corticosterone acetate; Dr. E. Schwenk of the Schering Corporation, Bloomfield, New Jersey, who supplied the progesterone and the ethinyl testosterone; and to Dr. M. Ehrenstein of this laboratory who prepared the sample of desoxy-corticosterone from its acetate and prepared the oil solutions of all of the test materials.

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THE WORK CAPACITY OF THE RAT IMMEDIATELY FOLLOWING PARTIAL ADRENALECTOMY

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THE CAPACITY of the rat for continued work of the gastrocnemius muscle can be used as an index of the functional adequacy of adrenal tissue and of the adequacy of replacement therapy in adrenalectomized animals. Ingle, Hales and Haslerud (1) have shown that the capacity to work may remain below normal when large amounts of adrenal cortical tissue are removed even after time is allowed for the regeneration and hyperplasia of the remaining tissue. Ingle (2, 3) has observed that much larger amounts of cortin are required for the maintenance of a normal capacity to work in adrenalectomized rats than are required for the maintenance of life and a state of apparent good health under optimal conditions. Horvath, Hitchcock and Hartman (4) have demonstrated that the unilaterally adrenalectomized rat is incapable of maintaining a normal resistance to cold. Hales, Haslerud and Ingle (5) reported that the work capacity of the unilaterally adrenalectomized rat was normal. This observation is not substantiated in the present work.

The object of this study was to determine the immediate effect of removing varying amounts of adrenal tissue upon the capacity of the rat to continue work. Comparisons were made among animals having (*a*), a sham operation, (*b*), a unilateral adrenalectomy and (*c*), a unilateral adrenalectomy with an additional partial ablation of the other adrenal. The removal of one adrenal had the effect of reducing the total amount of work performed by the animal; the removal of any additional amount of the remaining gland had the effect of further reducing the total amount of work performed.

METHODS

Male rats of the Sprague-Dawley strain which weighed approximately 180 gm. were used in these experiments. The animals were anesthetized with phenobarbital sodium. In 10 animals the adrenal glands were exposed but not damaged; in 26 animals both adrenals were completely removed; in 10 animals the right adrenal was removed and the opposite adrenal was left intact and in 28 animals the right adrenal was removed and varying amounts of the distal portion of the left adrenal were excised with a minimum of crushing of the remaining portion and without damage to the pedicle of the gland. Immediately following the operation one gastrocnemius muscle of each animal was weighted with 100 gm. and stimulated to contract 3 times per second until there was a complete loss of muscular responsiveness. Each animal received 5 cc. of water by subcutaneous injection at 11 A.M. and at 5 P.M. on each day that the test was continued. The details of the method have been described (3, 6). In this study the intensity of the faradic stimulation was increased over that used in previous studies.

RESULTS

The total amount of work performed by the individual animals of each experimental group (fig. 1) are presented graphically. The animals which had been subjected to false operations were able to work continuously for periods of 5 to 7 days. The

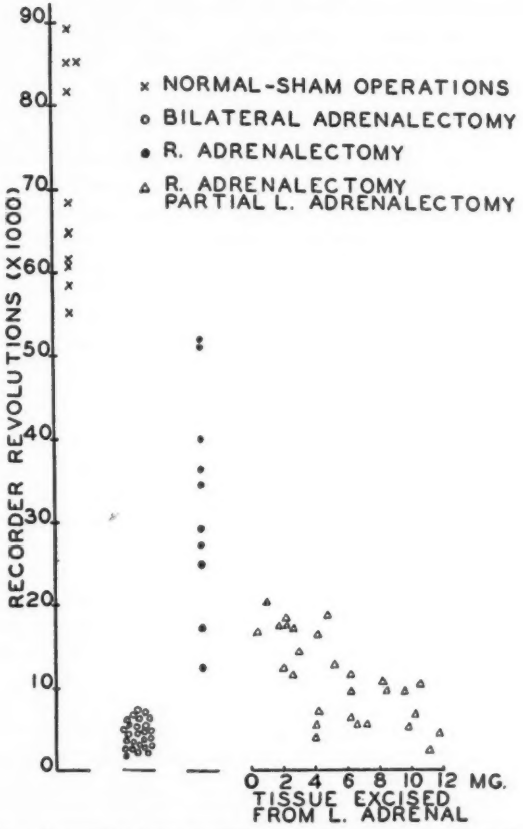

Fig. 1. Amount of work performed immediately following operation.

completely adrenalectomized animals all collapsed within the first 24 hours. The unilaterally adrenalectomized animals showed a decreased capacity to work as compared to the animals which possessed both adrenals. The partial ablation of the left adrenal in addition to the complete removal of the right adrenal decreased the capacity to work to an extent that was roughly proportional to the amount of tissue excised.

DISCUSSION

As judged by the effect of partial adrenalectomy on work, it can be concluded that the removal of even small amounts of tissue from the adrenal greatly decreases the functional capacity of the remaining portion of the gland. It is possible that part of the functional inadequacy results from damage to the circulatory mechanism of the remaining portion of tissue. Some functional activity was retained, however, since

the work performance of rats which had only small portions of the adrenal remaining performed greater amounts of work than did the completely adrenalectomized animals. In my opinion the smallest amount of tissue remaining would probably have been adequate to maintain life without evidence of adrenal cortical insufficiency under optimal living conditions (7). When the animals were subjected to the stress of these experimental conditions the functional inadequacy of the remaining cortical tissue was revealed. It is significant that the presence of one intact adrenal gland was not adequate to maintain a normal capacity for work under these conditions. This is in contrast to the previous findings of Hales, Haslerud and Ingle (5), regarding the work capacity of unilaterally adrenalectomized rats immediately after operation. The discrepancy can probably be explained by the fact that the intensity of the faradic stimulation used in the present experiment was increased over the intensity used in the earlier experiment, thus producing a more severe stress on the experimental animal. Horvath, Hitchcock and Hartman (4) have demonstrated that the unilaterally adrenalectomized rat is incapable of maintaining a normal resistance to cold.

In the clinical literature there are a number of case reports of patients who have manifested symptoms of adrenal insufficiency following sub-total removal of the adrenal cortices (8). Partial ablation of hyperplastic adrenals in man has been occasionally carried out in the absence of any other pathologic involvement of the gland. These procedures have been justified on the basis of the clinical improvement which follows their use. However, in animals, experimental enlargement of the adrenals is produced by some form of stress, and the hyperplastic gland is apparently one which has adapted itself to the physiologic requirement for its hormone. The removal of large amounts of the adrenal cortex may either be compensated for through regeneration (7), or if the damage has been too extensive the cortex may never regenerate to its former size (7) and may not regain an adequate functional capacity (1).

The writer has conducted studies (2, 3, 9) on completely adrenalectomize drats with the objective of maintaining a normal resistance of such animals to severe stress by treatment with cortin and cortin-like substances. The difference between the amounts of the hormone which are required to maintain life and apparent normality under non-stress conditions and the amounts which are required to maintain a normal resistance to stress is very large and suggests that some qualitative deficiency in the hormone is involved. Although this question cannot yet be answered it is significant that a very wide difference also exists between the amounts of adrenal tissue which will maintain life and the amounts required to maintain a normal resistance to stress.

SUMMARY

Partially adrenalectomized male rats were studied in respect to their capacity to continue work of the gastrocnemius muscle. The removal of one adrenal had the effect of reducing the total amount of work below that of the sham operated animals; the removal of any additional amount of the remaining gland had the effect of further reducing the total amount of work performed.

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ON THE MODE OF ACTION OF CALCIFEROL IN THE NEPHRECTOMIZED RAT

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THE OBJECT of the present paper is to show the effect of massive doses of calciferol on the serum calcium of rats after they were subjected to simultaneous thyroparathyroidectomy and nephrectomy, and to show the effect of calciferol in nephrectomized rats which were thyroparathyroidectomized 7 days before they were nephrectomized. These data are correlated with previous observations (1, 2) and an explanation of the mode of action of calciferol in the nephrectomized rat is presented.

EXPERIMENTAL

The methods used in these experiments have been fully described in connection with closely related experiments that have been reported in previous publications (1, 2).

Table 1 indicates that calciferol was an effective factor in maintaining the serum calcium level during a post-operative period of approximately 48 hours which followed the double operation of thyroparathyroidectomy and bilateral nephrectomy. In none of the injected animals (*Rats* 1–8) did the serum calcium fall below normal values, and in 2 of the animals (*Rats* 1 and 2) it increased markedly. The serum calcium of the uninjected animals (*Rats* 9–12) showed the same variable tendency to decrease that we have observed repeatedly in the rat with intact kidneys during the first 48 hours after thyroparathyroidectomy. Histological examination of the femurs of the injected animals (*Rats* 1–4) revealed in the first 3 animals a slight to moderate increase in the osteoclast action, but the femur of *Rat* 4 appeared to have been unaffected by the calciferol.

Six rats (table 2), which were thyroparathyroidectomized 7 days prior to being nephrectomized, were maintained on our stock diet up to the time of nephrectomy when the food was withdrawn. As will be noted, the injection of calciferol appears to have had no effect on the serum calcium in these animals. Histological examination of the femurs of *Rats* 1, 2, 4 and 5 revealed a slight increase in the osteoclast action in *Rats* 1 and 2, but the bone of *Rat* 4 was normal in appearance The femur of the control animal (*Rat* 5) also exhibited no evidence of resorption.

DISCUSSION

In previous work (1), McJunkin, Tweedy and McNamara observed that the administration of single subcutaneous doses (460,000 I.U.) of calciferol to young normal rats, from which food was withheld during a 48-hour experimental period, gave either doubtful or indecisive increases in the serum calcium. They also observed that the femurs of these animals were devoid of any distinct osteoclastic resorption. Even though massive doses (3 × 460,000 I.U.) of calciferol were injected subcutaneously in divided doses into normal rats no osteoclastic resorption was produced. Furthermore these authors detected no deleterious effects of calciferol on the general health of their animals. It appears to us that the negative results, obtained during the short experi-

mental period, may have been due to the slow assimilation of calciferol by the subcutaneous route of injection and the absorption of calcium which was present in the intestines of the animals at the time the injections were begun.

Shelling (3) has shown that large doses of viosterol (20,000%) fed over a period of 5 to 25 days to young normal rats, which were kept on a calcium- and phosphorus-free diet, produced a moderate or pronounced hypercalcemia (12.9 to 19.7 mg.%) and slight to moderate metastatic calcification. In some of his animals he observed slight to moderate osteoporosis. Most of his animals developed diarrhea and lost weight soon after the viosterol feeding was begun.

In our first work (4) with the untreated nephrectomized rat we found that the serum calcium remained within normal limits, or fell very gradually, during a post-operative period of 48 hours, even though the serum inorganic phosphorus might exceed 20 mg.% during the same period (5). Histological examination of the femurs of these animals revealed no evidence of osteoclastic resorption (1). It would seem that actively secreting parathyroid glands are responsible for the maintenance of normal serum calcium values in the nephrectomized rat, and that acidosis is probably a minor factor in its effect on the serum calcium level in this animal, since in animals subjected to simultaneous thyroparathyroidectomy and nephrectomy (*Rats* 9–12, table 1) we find that the serum calcium exhibits the same tendency to decrease that is seen after thyroparathyroidectomy alone. Furthermore the observations of Jarrett, Peters and Pappenheimer (6) on the partially nephrectomized rat suggest the possibility that the parathyroid glands may be stimulated to hypersecretion by the effects arising from bilateral nephrectomy. These investigators have reported that unilateral nephrectomy and partial destruction of the remaining kidney in the rat leads within several days to a measurable increase in the size of the nuclei and the cytoplasmic volume of the parathyroid cells.

TABLE 1. EFFECT OF CALCIFEROL ON THE SERUM CALCIUM LEVEL OF RATS AFTER SIMULTANEOUS THYROPARATHYROIDECTOMY AND NEPHRECTOMY

Rat No.[1]	Weight	Calciferol[2] injected	Serum calcium
	gm.	I.U.	*mg./100 cc.*
1	127	460,000	15.78
2	165	460,000	17.78
3	190	460,000	10.60
4	177	2×460,000	12.86
5	172	3×460,000	13.05
6	248	3×460,000	10.26
7	189	3×460,000	10.61
8	220	3×460,000	11.50
9	176	—	8.50
10	189	—	5.66
11	218	—	5.66
12	180	—	10.69

[1] *Rats* 6, 8 and 11 were litter mates, as were also *Rats* 5, 7 and 10. Equal parts of blood serum obtained from *Rats* 10 and 11 were blended and analyzed for calcium.

[2] The calciferol was dissolved in fresh corn oil and administered subcutaneously. *Rats* 1 and 3 each received a single injection of calciferol 1 hour after operation, but calciferol was not administered to *Rat* 2 until 18 hours after the operation. The multiple injections were made at 1, 6 and 18 hours after nephrectomy.

McJunkin, Tweedy and McNamara (1) have shown that unlike the normal rat, the nephrectomized rat with intact parathyroids is quickly affected by the subcutaneous injection of massive doses (3×460,000 I.U.) of calciferol. Within 48 hours there was a slight to a marked increase in the osteoclast action in most of the animals and a

concomitant increase in the serum calcium to hypercalcemic levels (above 15 mg.%). In view of their failure to obtain hypercalcemia by the injection of large doses of parathyroid hormone in the nephrectomized rat, they were of the opinion that the hypercalcemia obtained by injection of calciferol was due to the action of the latter substance alone, unaided by the activity of the animal's own parathyroid glands. In the light of subsequent experiments, however, we are of the opinion that the actively secreting parathyroid glands of the animal played a part in the production of the hypercalcemia.

It is seen in table 1 that after simultaneous removal of the parathyroids and the kidneys, hypercalcemia occurred in only 2 of the 8 animals which were injected with calciferol. Calciferol appears to have been largely responsible for the maintenance of a normal, or slightly elevated, serum calcium level in 6 of the animals, and in 2 of the animals the serum calcium values indicate that calciferol was an effective factor in the production of hypercalcemia. It is quite possible that in some of these animals preformed parathyroid hormone was present in sufficient amount to have promoted the intake of the excess calcium from the bone into the blood.

TABLE 2. EFFECT OF CALCIFEROL ON THE SERUM CALCIUM LEVEL IN THYROPARATHYROIDECTOMIZED-NEPHRECTOMIZED RATS

Rat No.[1]	Weight	Calciferol injected	Serum calcium
	gm.	I.U.	*mg./100 cc.*
1	220	3×460,000	8.56
2	144	3×460,000	6.90
3	190	3×460,000	4.21
4	143	3×460,000	8.73
5	232	—	4.93
6	128	—	8.38

[1] *Rats* 1, 3 and 5 were litter mates as were also *Rats* 2, 4 and 6.

Seven days after thyroparathyroidectomy, injection of massive doses of calciferol failed to produce hypercalcemias in our nephrectomized rats (table 2). However, an increase in the osteoclast action in 2 of the animals (*Rats* 1 and 2) indicates that a disturbance in bone metabolism had been induced. It would appear that insufficient time had elapsed after thyroparathyroidectomy for the bones of these animals to have become depleted of available calcium, so we are inclined to attribute the failure of calciferol to produce hypercalcemia in these animals (table 2) to a greater imbalance in the serum calcium and inorganic phosphorus, and consequently a slower inflow of bone calcium into the blood than occurs in the nephrectomized rat with intact parathyroids.

Recently, we reported (2) that neither an increase in serum calcium nor an increase in osteoclast action was detectable in a series of 15 rats which were thyroparathyroidectomized and kept on our stock diet for 30 days before they were nephrectomized and injected with calciferol (3 to 6×460,000 I.U.). That such animals are not insensitive to the action of calciferol, was shown by the following experiment. A group of 18 rats with 19 controls were treated in a similar manner to the animals just mentioned, except that they were maintained on a high calcium-low phosphorus diet during the 30-day interval between thyroparathyroidectomy and nephrectomy. The 11 animals of the first group, which remained on the above diet until they were nephrectomized and injected with calciferol, showed a marked increase in serum calcium, which in two instances reached hypercalcemic levels. Supplementary data obtained in the above experiment strongly indicate that the rise in serum calcium was due to the action of calciferol in restricting the fecal excretion of calcium.

From the foregoing experiments it appears that calciferol does not act directly upon the bone of the nephrectomized rat to release calcium to the blood. The action of calciferol seems to be limited to the bowel, where it restricts the fecal excretion of calcium that enters the blood from the bone, or by absorption from the intestines. In the absence of both the parathyroid hormone and the kidneys, the high serum inorganic phosphorus that is present in such animals is probably not conducive to the rapid intake of bone calcium by the blood. Consequently, unless a considerable amount of calcium enters the blood by absorption from the intestine, hypercalcemia is not produced in the nephrectomized animal by calciferol.

Although Shelling (3) has shown that large doses of viosterol have the effect of directing the excretion of calcium and phosphorus through the kidneys of the normal rat that is kept on a calcium- and phosphorus-free diet, he appears to attribute the resorption of bone and the hypercalcemia, which he observed (7) in the parathyroidectomized rat, solely to a direct action of viosterol on the bone. Recently Clémens (8) has reported that the urinary excretion of phosphates by dogs was greatly increased when they received daily (4 to 25 days) subcutaneous injections of irradiated ergosterol (75 to 400,000 I.U. per kg. body wt.). He found that when the kidneys of a treated dog, and the kidneys of an untreated normal dog, were grafted into the neck of a normal dog, the kidneys of the first excreted twice the amount of phosphates excreted by the kidneys of the second, and many times the amount of phosphates excreted by the intact kidneys of the normal dog. Clémens is of the opinion that irradiated ergosterol has a direct action upon the kidney by which the renal threshold for phosphates is lowered. From our observations on the action of calciferol in the nephrectomized rat, it appears to us that the resorption of bone in the parathyroidectomized rat with intact kidneys could be produced through the action which viosterol exerts upon the escretion of calcium and phosphorus.

SUMMARY

The subcutaneous administrative of massive doses of calciferol prevents the fall in serum calcium which usually occurs after thyroparathyroidectomy in the nephrectomized rat. Calciferol produces no observable effect on the serum calcium of the nephrectomized rat that is thyroparathyroidectomized 1 week prior to nephrectomy. The action of calciferol appears to be upon the bowel where it decreases the fecal excretion of calcium.

The calciferol used in these experiments was generously supplied by Mead Johnson and Company through the courtesy of Dr. C. E. Bills.

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THE EFFECT OF THE THYROTROPIC PRINCIPLE OF THE ANTERIOR PITUITARY ON THE THYROID OF THE UNDERNOURISHED GUINEA PIG

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PREVIOUS INVESTIGATORS have shown that prolonged inanition in the experimental animal is accompanied by morphological alterations in the thyroid gland, characterized by atrophy, involution and degeneration (1, 2). Myers (3) and Stefko (4) have described similar changes in man in undernutrition. More recently Rabinovitch (5) has described the thyroid of guinea pigs fed an inadequate diet resulting in the loss of 20 to 30% of their weight in 9 to 11 days. In such animals there was almost complete cessation of proliferative activity, the acinar epithelium was flattened and the colloid was hard and dense. There is some evidence (6, 7) that certain of the vitamin deficiencies may result in structural changes in the thyroid similar to those which have been described in inanition.

The present investigation, in which the observations of Rabinovitch have been confirmed, is concerned chiefly with two aspects of the problem: *a*), the effect of feeding of vitamin supplements on the morphological changes associated with inanition and *b*), The effect of the thyrotropic principle of the anterior hypophysis on the thyroid of the undernourished guinea pig.

Female guinea pigs, weighing between 250 and 275 gm. were kept under observation in the laboratory on a standard diet of oats, hay and lettuce until they had gained in weight to approximately 300 gm. For the underfeeding experiments the animals were placed in individual cages and fed once daily. Water was available at all times. A modified Sherman diet[1] was used. In the preparation of the diet heating of the skim milk powder was omitted. When prepared in this fashion, the diet is not scorbutic for the guinea pig (8); animals killed after 3 weeks on this diet have shown no evidences of scurvy. Five grams of this diet was fed once daily as the basal ration during the periods of undernutrition. On such a diet the female guinea pig weighing 300 gm. loses from 20 to 30% of original weight in a period of 2 weeks. At the conclusion of the experimental period, the animals were killed, the thyroids and ovaries were cleanly removed, weighed immediately and fixed in Zenker's solution. The organs were imbedded in paraffin, sectioned and stained with hematoxylin and eosin. Observations on the ovaries of these animals will be described in a separate communication. In the experiments in which the thyrotropic principle of the anterior hypophysis was used, the extract was injected for 4 consecutive days; the animal was killed on the fifth day. A total of 110 animals, divided into several groups as described below, were used in this investigation. Weight curves and photomicrographs of the

[1] The composition of the diet was as follows: wheat bran, 29.5 parts; rolled oats, 29.5 parts; skim milk powder, 30 parts; sodium chloride, 1 part; butter fat, 8 parts; cod liver oil, 2 parts.

thyroid glands of representative animals of various groups are shown in the accompanying chart and figures.

The morphology of the thyroid of normal guinea pigs studied in this laboratory is similar to that described by others. In the healthy animal which is gaining weight, the acini are lined by cuboidal epithelial cells, with round nuclei and a moderate amount of cytoplasm (fig. 1). The colloid shows varying degrees of peripheral vacuolization and irregular retraction from the acinar wall, interpreted as evidence of liquefaction and active absorption of colloid. Occasionally mild degrees of relative hyper-

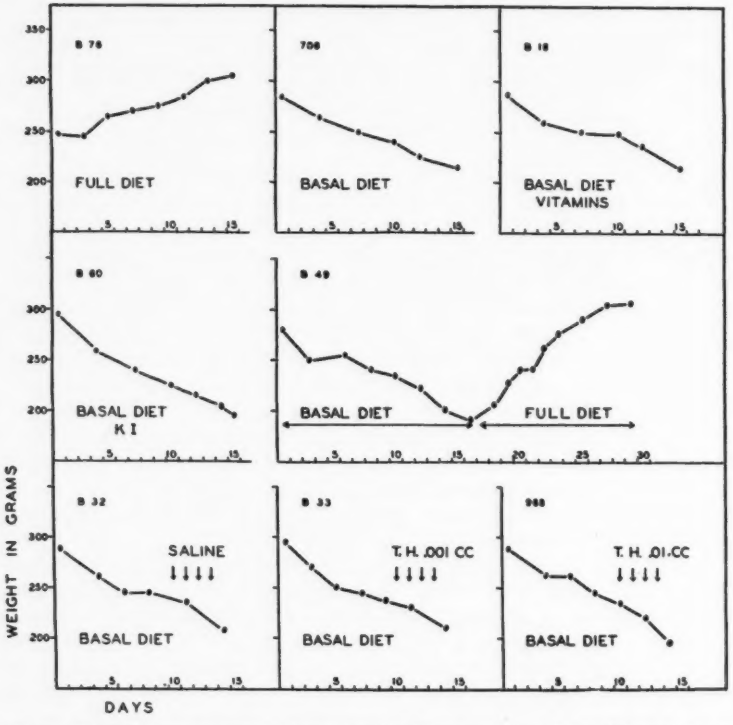
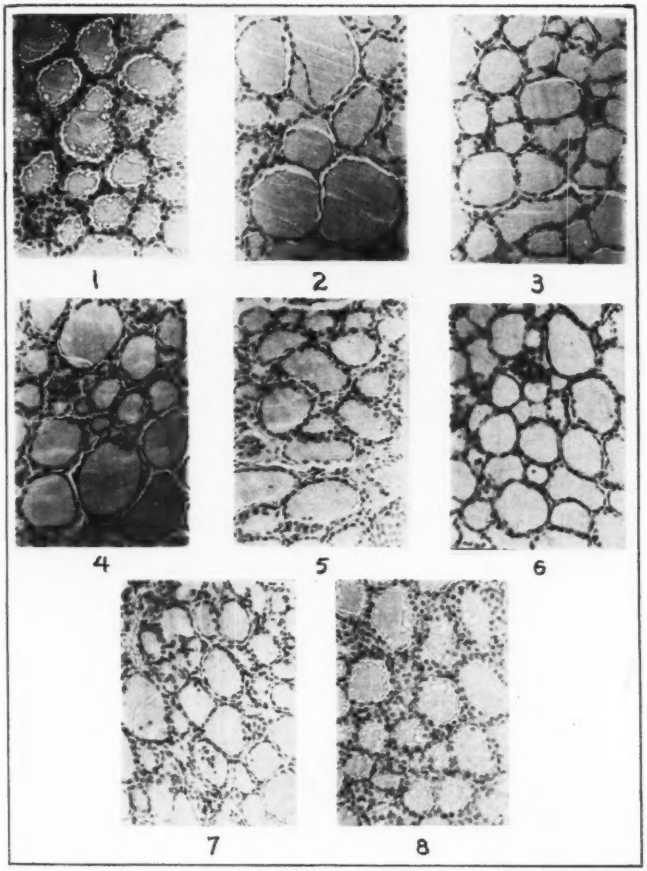


Chart 1. Weight curves of representative animals. Experimental conditions of the various groups are summarized in the text and in the explanation of the figures. Photomicrographs of the thyroid glands of the animals depicted above are shown in figures 1, 2, 5, 6, 8, 7, 13 and 16.

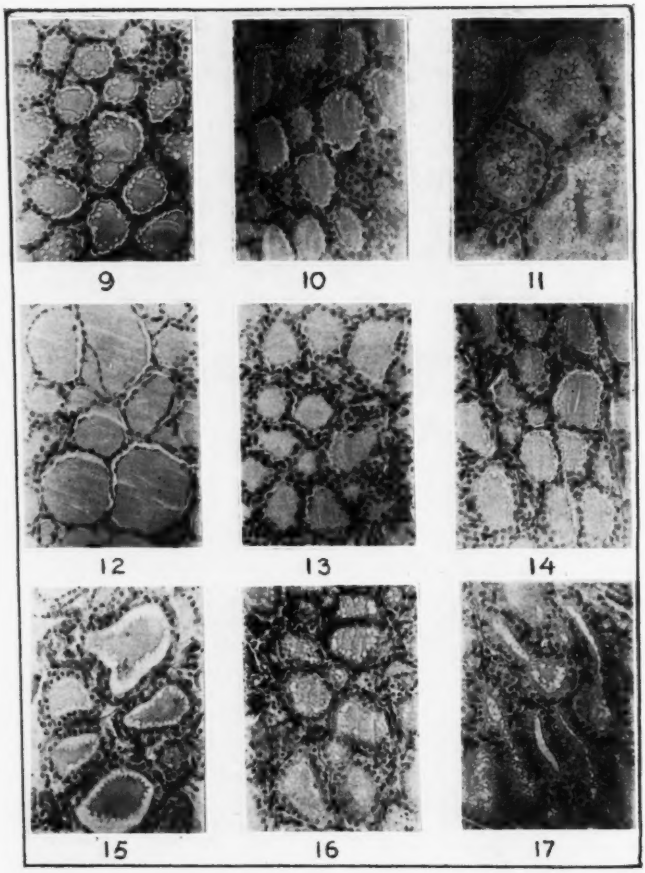
plasia or involution are observed in some parts of the glands of normal animals. It has been found that changes in the environment, or in the diet, of the normal guinea pig may be associated with temporary loss or failure to gain in weight, with modification of the histological picture of the thyroid. For this reason, only animals which were gaining in weight at the time were utilized as controls and for the underfeeding experiments.

Animals which had lost from 20 to 30% of body weight in a period of 2 weeks (chart 1) showed striking structural changes in the thyroid glands. The acinar epithelial cells were changed from cuboidal to an extremely flat type (fig. 2, 3). In most instances, only a thin, hair-like line of epithelium remained around the distended



PHOTOMICROGRAPHS OF THYROID GLAND, ×125. Fig. 1. G.P. *B*-76. Normal guinea pig. Weight 305 gm. Fig. 2. G.P. 706. Undernourished guinea pig. Initial weight 280 gm. Final weight 215 gm. Basal diet Fig. 3. G.P. 708. Undernourished guinea pig. Initial weight 325 gm. Final weight 245 gm. Basal diet. Fig. 4. G.P. *B*-16. Undernourished guinea pig. Initial weight 285 gm. Final weight 205 gm. Basal diet plus 2 mg. ascorbic acid daily. Fig. 5. G.P. *B*-5. Undernourished guinea pig. Initial weight 300 gm. Final weight 235 gm. Basal diet plus 2 mg. ascorbic acid, 1 cc. cod liver oil and 1 yeast tablet containing 25 I.U. vitamin B_1 and 97 riboflavin daily. Fig. 6. G.P. *B*-60. Undernourished guinea pig. Initial weight 290 gm. Final weight 210 gm. Basal diet plus 50 mg. KI daily. Fig. 7. G.P. *B*-32. Undernourished guinea pig. Initial weight 285 gm. Final weight 210 gm. Basal diet. Intraperitoneal injections of 0.5 cc. physiological saline on the 10, 11, 12, 13 days, killed on the 14th day. Fig. 8. G.P. *B*-49. Re-fed guinea pig. Initial weight 275 gm. Basal diet 15 days, weight 220 gm. Then unlimited diet for 14 days. Final weight 305 gm.

follicular contents. The cytoplasm of the acinar epithelial cells was inconspicuous and frequently appeared to be absent, with only the nucleus and cell membrane remaining. The nuclei were small, elongated, densely stained and granular. Mitotic figures and nucleoli were absent. The acini were filled with dense staining, eosinophilic colloid. Vacuolization and retraction of the colloid were absent and there were no evidences of softening or absorption of colloid. The general picture was that of an inactive, resting gland, with flat, atrophic epithelium lining acini which were dis-



PHOTOGRAPHS OF THYROID GLAND, ×125. Fig. 9. *G.P. B-76.* Normal guinea pig. Weight 305 gm. Fig. 10. *G.P. B-41.* Normal guinea pig. Weight 300 gm. Intraperitoneal injections of 0.01 cc. thyrotropic hormone for 4 consecutive days, killed on the 5th day. Fig. 11. *G.P. B-42.* Normal guinea pig. Weight 295 gm. Intraperitoneal injections of 0.02 cc. thyrotropic hormone for 4 consecutive days, killed on the 5th day. Fig. 12. *G.P. 706.* Undernourished guinea pig. Initial weight 280 gm. Final weight 215 gm. Basal diet. Fig. 13. *G.P. B-33.* Undernourished guinea pig. Initial weight 295 gm. Final weight 210 gm. Basal diet. Intraperitoneal injections of 0.001 cc. thyrotropic hormone on the 10, 11, 12, 13th days, killed on the 14th day. Fig. 14. *G.P. B-36.* Undernourished guinea pig. Initial weight 295 gm. Final weight 220 gm. Basal diet. Intraperitoneal injections of 0.002 cc. thyrotropic hormone on the 10, 11, 12, 13th days, killed on the 14th day. Fig. 15. *G.P. B-23.* Undernourished guinea pig. Initial weight 270 gm. Final weight 195 gm. Basal diet. Intraperitoneal injections of 0.005 cc. of thyrotropic hormone on the 10, 11, 12, 13th days, killed on the 14th day. Fig. 16. *G.P. 968.* Undernourished guinea pig. Initial weight 280 gm. Final weight 195 gm. Basal diet. Intraperitoneal injections of 0.01 cc. thyrotropic hormone on the 10, 11, 12, 13th days, killed on the 14th day. Fig. 17. *G.P. 495.* Undernourished guinea pig. Initial weight 275 gm. Final weight 220 gm. Basal diet. Intraperitoneal injections of 0.02 cc. thyrotropic hormone on the 10, 11, 12, 13th days, killed on the 14th day.

tended with dense colloid. These changes were consistent, and were uniform throughout the gland. In the normal thyroid of the guinea pig, acini similar to those described in the undernourished animals are often seen in the extreme periphery of the gland but have not been observed in central portions of the thyroids of healthy

animals which are gaining weight at the time of being killed. The observations described and the photomicrographs shown in the figures were always made in central portions of the gland. The above findings are similar to those of Rabinovitch (5), who has suggested that the reduction in metabolism which occurs in the case of general undernutrition may be due, in part at least, to the changes which take place in the thyroid as a result of underfeeding.

A group of animals was underfed for a period of 2 weeks and then given a full diet in unlimited amounts. Such animals rapidly regained the weight that had been lost (chart 1). Examination of the thyroids of animals which had been re-fed revealed an essentially normal structure (fig. 8). The acinar epithelium had returned to the cuboidal type and there was vacuolization and peripheral retraction of the colloid similar to that seen in the normal gland (fig. 1).

In view of the complicated interrelationships between the various vitamins and the endocrine glands (9), it was considered advisable to investigate the effect of feeding vitamin supplements on the structural changes in the thyroids of the undernourished animals. One group of guinea pigs was given lettuce daily in addition to the basal ration. Another group was given 2 mg. of ascorbic acid in the form of an orange juice powder. In a third group of animals, the basal diet was supplemented by a daily ration of 2 mg. of ascorbic acid, 1 cc. of cod liver oil, and one yeast tablet containing 25 I.U. of vitamin B_1 and 9γ of riboflavin. In these animals the rate of weight loss (Chart 1) and the structural changes in the thyroid (fig. 4, 5) were similar to those observed in the undernourished animals fed the basal diet without vitamin supplements. From these observations it was concluded that the changes in the thyroid glands of the undernourished animals were not due primarily to vitamin deficiency.

In the normal guinea pig the administration of small doses of potassium iodide results in stimulation and proliferation of the acinar epithelium (10). Rabinovitch and Gray (11) have shown that the mitotic cell proliferation which occurs normally in the thyroid of the guinea pig after the administration of potassium iodide may be completely inhibited by underfeeding. This observation has been confirmed in the present study. A group of animals received 50 mg. of potassium iodide daily during the period of underfeeding. There was no effect on the rate of weight loss (Chart 1) and the structural changes in the thyroid were not modified (fig. 6).

Studies of the effect of the thyrotropic principle of the anterior hypophysis were stimulated by the observation that relatively large amounts of the principle, which were tolerated without difficulty by normal animals, resulted in death within 24 hours when administered to undernourished animals. Further studies showed that morphological changes could be demonstrated in the thyroid glands of the undernourished animals with amounts of the thyrotropic principle which were much smaller than the minimal effective dose for the normal guinea pig.

The anterior pituitary extract[2] used in this study was assayed by the manufacturer to contain from 50 to 100 guinea pig units of the thyrotropic principle per cc. Saline dilutions of this extract were prepared for injection; the dosages indicated in the text, chart and in the figures are expressed in amounts of the undiluted extract. Figures 10 and 11 show the effect on normal female guinea pigs, weighing approximately 300 gm., of 0.01 and 0.02 cc. respectively, injected for 4 consecutive days. The smaller dose resulted in minimal evidences of stimulation in a few animals but in most instances there was no detectable change in the morphology of the gland. Normal animals receiving 0.02 cc. uniformly showed evidences of marked thyroid stimulation (fig. 11). For this preparation, used in this manner, 0.01 cc. was appar-

[2] The antuitrin-T used in this study was generously provided by Parke, Davis & Company.

ently the threshold level for animals of this sex and weight. In another series of experiments not recorded here, approximately the same results were obtained when the customary assay animal, the male guinea pig weighing from 150 to 200 gm., was used.

The effect of graded doses of the thyrotropic principle, ranging from 0.001 cc. to 0.02 cc. was studied in groups of undernourished animals. The administration of 0.5 cc. of physiological saline for 4 consecutive days to a group of undernourished animals was shown to be without effect (fig. 7). The smallest dose of thyrotropic principle used, 0.001 cc. injected for 4 consecutive days, resulted in a definite modification of thyroid structure (fig. 13) as compared with the untreated animals (fig. 12) and with the saline injected animals (fig. 7). This was noted particularly in a definite increase in the height of the acinar epithelial cells, with increase in cytoplasm, rounding out of the nucleus and the appearance of mitotic figures, but with little change in the appearance of colloid. With increasing doses of thyrotropic principle, increasing evidences of thyroid stimulation could be seen. The next dose, 0.002 cc., resulted in further increase in height of the epithelial cells and beginning vacuolization and retraction of the colloid (fig. 14). Further evidences of epithelial stimulation and colloid softening were apparent with the administration of 0.005 cc. and 0.01 cc. (fig. 15, 16). Maximal stimulation, with almost complete resorption of colloid, slit-like acini and marked proliferation of acinar epithelium were seen with doses of 0.02 cc. of thyrotropic principle (fig. 17).

These observations indicate that the thyroid of the undernourished guinea pig is markedly sensitive to the thyrotropic principle of the anterior hypophysis and that structural changes in the glands of such animals may be demonstrated with as little as one-tenth of the minimal effective dose for normal animals. The possibility of using the undernourished guinea pig for the detection of small amounts of thyrotropic hormone is suggested by these findings. The Collip rat unit, which requires an hypophysectomized animal, compares fairly well with the Junkmann-Schoeller unit as determined by the normal guinea pig (12). The chick unit of Bergman and Turner (13) is estimated to be about one-fourth of the conventional guinea pig unit. If these comparisons are valid, the use of the undernourished guinea pig may prove to increase the sensitivity of the biologic assay methods now in use for the detection and estimation of the thyrotropic principle. Further investigation of this phase of the problem is in progress.

The results of this investigation suggest a possible explanation for the structural changes, and perhaps the reduction in basal metabolism, which have been observed in undernutrition. In many ways, the observations here recorded are similar to those which have been described in hypophysectomized animals. Thyroid atrophy, with flattening of acinar epithelium and retention of colloid as observed in the undernourished animal is very similar to the structural changes induced in the thyroid by hypophysectomy (14). Anderson and Collip (15) have shown that hypophysectomy markedly increases the sensitivity of the rat to thyrotropic hormone; the present investigation shows that a similar increase in sensitivity to pituitary stimulation occurs in the thyroid of the undernourished guinea pig. Another clue may be found in the lack of response of the thyroid of the undernourished animal to potassium iodide; Loeser and Thompson (16) have shown that the stimulating effect of iodine on the thyroid is abolished by hypophysectomy.

It is of interest to note that there is also evidence of inhibition of the gonadotropic principle of the anterior pituitary in undernutrition. Moore and Samuels (17) observed that partial inanition caused changes in the rat prostate and seminal vesicles

similar to those found in the castrate animal. These changes were promptly corrected not only by testis hormone by also by the administration of the pituitary-like principle obtained from pregnancy urine, indicating that the testis was responsive to stimulation and suggesting that suppression of the hypophyseal gonadotropic principle was responsible for the deficiency of testis hormone. In undernourished animals studied by Selye and Collip (18), ovarian atrophy was observed; the formation of normal corpora lutea followed the administration of the pregnancy urine principle. These investigators concluded that under conditions of malnutrition, the pituitary secretes less of the gonadotropic principle but that it remains fully responsive to such stimuli as the anterior-like principle of pregnancy urine. Similarly Kurzrok and his associates (19) have attributed the anestrus of undernourished rats to diminished production of the hypophyseal gonadotropic hormone. More direct evidence of pituitary involvement in undernutrition is to be found in the observations of Mason and Wolfe (20), who reported that the hypophyses of animals upon an inadequate diet were lower in sex stimulating capacity than those from normal animals.

There is, then, a considerable body of evidence that there is suppression of anterior pituitary function in the undernourished animal. It is postulated that the striking structural changes observed in the thyroid gland in inanition may be due to decreased secretion of the pituitary thyrotropic principle, upon which the normal thyroid is dependent for maintenance of normal structure and function. Evidence in favor of this hypothesis is to be found in the present study in *a*), the similarity of thyroid structure of the undernourished animals to that of the hypophysectomized animal and *b*), the marked increase in sensitivity of the thyroid of the undernourished animal to pituitary stimulation and the refractiveness to stimulation by potassium iodide, both of which are characteristic of animals in which the pituitary has been removed.

SUMMARY

Undernutrition in the guinea pig, of sufficient degree to cause a loss of 20 to 30% of body weight in a period of 2 weeks, results in marked structural changes in the thyroid, characterized by atrophy and flattening of the acinar epithelium and retention of colloid, suggesting a resting, inactive gland. These changes are not affected by the administration of vitamin supplements or by the administration of potassium iodide. Re-feeding results in a rapid gain in weight and return of thyroid structure to normal.

Thyroid of the undernourished guinea pig shows a marked increase in sensitivity to the thyrotropic principle of the anterior hypophysis. In such animals structural changes indicate that the thyroid may be stimulated by the injection of as little as one-tenth the minimal effective dose for normal animals.

It is suggested that the use of the undernourished guinea pig may increase the sensitivity of present methods for the detection and estimation of small amounts of the thyrotropic hypophyseal principle.

Evidence is presented which suggests that suppression of anterior pituitary function occurs in inanition. It is postulated that the structural changes observed in the thyroid in undernourished animals may be due to decreased secretion of the pituitary thyrotropic principle. Support for this hypothesis is to be found in the similarity of thyroid structure of the undernourished animal to that of the hypophysectomized animal, the marked increase in sensitivity of the thyroid in inanition to pituitary stimulation and in the absence of the stimulating effect of potassium iodide.

The author wishes to extend his thanks to Mr. Frank Smith, who assisted in the preparation of the photomicrographs.

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LIVER GROWTH IN RATS TREATED WITH ANTERIOR PITUITARY GROWTH HORMONE

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TREATMENT WITH ANTERIOR PITUITARY GROWTH HORMONE or its overproduction by the gland causes a relatively greater growth of the visceral and glandular organs than of the other body tissues (1, 2). Conversely, splanchnomicria is regularly found in pituitary dwarfism, and atrophy of the visceral organs characteristically ensues after hypophysectomy in animals (3). In rats treated with A P growth principle the liver shows a relatively greater growth response than most of the other visceral organs or the body as a whole (1, 4). The experiments reported below were undertaken to determine the histological character of this hepatic growth response to anterior pituitary treatment. The primary question was whether the cellular elements are increased in number or in size.

METHODS

The animals used were 54 female rats (Wistar strain), weighing between 175 and 224 gm. and 3 to 8 months of age. These were grouped into pairs or trios, the members of each pair or trio being litter mates and weighing the same within a few grams at the start. The paired-feeding method (5) was used throughout and paired feeding was started several days before choosing the animals for their respective groups. By this method the two members of each pair were allowed to consume exactly the same amount of food daily as determined by the appetite of the less hungry member. Of each pair, one rat was then selected as the control and the other was treated with growth hormone[1] (2 units twice daily), for periods ranging from 1 to 16 days. In the trios, one rat served as the control, the second was treated and kept on paired feeding and the third was treated but allowed food ad libitum. In selecting the groups the more hungry rat of each pair was made the control. (Immediately after treatment, in the groups kept for 3 days or longer, this condition was shifted and the treated member in nearly every daily instance became the more hungry, although the amount of food allowed was the same except in the case of trios in which the third member was unrestricted.)

In all cases food was withheld for 16 hours before the animals were killed. Immediately after death the livers were removed, exsanguinated by gentle manipulation in absorbent paper, samples removed and weighings made. Samples were always removed from the main lobe of the liver, across the middle portion, in the form of a slice about 2 mm. thick. They were fixed in neutral formalin. From this point on the specimens were treated exactly according to the procedure described by Brues, Drury and Brues (6), and counts of hepatic cell nuclei were made according to their technic. It was assumed that all hepatic cells were mononuclear. A sample protocol follows.

[1] The growth hormone used was a purified extract of beef anterior lobe, prepared and assayed by ourselves.

TABLE 1. BODY AND LIVER WEIGHTS, AND HEPATIC CELL ESTIMATIONS

Group	Controls—Paired Feeding						Growth Hormone Treated—Paired feeding							Growth Hormone Treated—Food ad libitum					
	Final body wt.	Gain in body wt.	Liver wt.	Ratio L.W./ B.W.	Hepatic cells	Hepatic cells/gm. liver	Days treated	Fina body wt.	Gain in body wt.	Liver wt.	Ratio L.W./ B.W.	Hepatic cells	Hepatic cells/gm. liver	Final body wt.	Gain in body wt.	Liver wt.	Ratio L.W./ B.W.	Hepatic cells	Hepatic cells/gm. liver
	gm.	gm.	gm.	%	(×10⁶)	(×10⁶)		gm.	gm.	gm.	%	(×10⁶)	(×10⁶)	gm.	gm.	gm.	%	(×10⁶)	(×10)⁶
1	208	−2	6.25	3.00	709	113	1.0	211	3	7.84	3.71	960	122						
2	205	5	8.01	3.90	966	121	1.3	205	11	7.99	3.89	942	118						
3	191	1	7.92	4.14	1047	132	1.3	203	13	7.09	3.49	893	126						
4	180	−6	6.61	3.67	712	108	1.5	182	5	6.50	3.57	712	109						
5	191	−6	5.56	2.91	688	124	2.0	201	4	7.32	3.64	738	101						
6	178	0	6.20	3.48	641	103	2.0	190	16	7.39	3.88	778	105						
7	222	2	6.05	2.72	727	120	2.5	228	11	8.28	3.63	794	95						
8	181	−5	6.54	3.61	662	101	2.5	194	7	9.55	4.92	1248	130						
9	205	−3	5.97	2.91	748	125	3.0	215	8	8.50	3.95	994	117						
10	206	−3	6.83	3.31	753	110	3.0	222	15	8.06	3.63	872	108						
11	212	−6	7.40	3.49	931	126	4.0	233	15	7.22	3.09	870	120						
12	209	1	7.54	3.60	775	103	4.0	229	23	9.66	4.21	1102	93						
13	216	−8	7.11	3.29	738	104	5.0	237	16	9.49	4.00	778	82						
14	216	−8	8.48	3.92	856	101	6.0	239	18	9.28	3.88	918	99						
15	197	5	6.29	3.19	680	108	6.0	216	23	7.30	3.37	741	101						
16	216	4	7.31	3.38	1108	152	16	241	31	8.86	3.67	1350	152	242	32	8.69	3.59	953	110
17	210	2	7.02	3.34	835	119	16	228	23	8.94	3.91	1102	123	248	45	10.37	4.18	1308	126
18	193	−5	6.23	3.22	919	116	16	211	23	6.29	2.98	815	129	218	34	7.63	3.50	796	104
19	202	0	6.59	3.26	854	130	16	229	22	7.49	3.27	1079	144	236	35	8.58	3.63	771	90
20	202	4	6.46	3.20	753	116	16	213	29	6.79	3.18	829	122	237	46	9.81	4.13	958	98
21	191	9	5.87	3.07	662	113	16	201	27	7.66	3.81	908	118	232	56	9.09	3.87	990	109
22	205	5	7.06	3.44	719	102	16	220	27	8.56	3.89	802	94	227	32	8.31	3.66	814	98
23	193	2	6.12	3.17	684	112	16	207	28	10.71	5.17	1116	113	223	37	8.60	3.85	801	93
Mean	201	−0.5	6.76	3.36	790	116		215	17	8.12	3.77	928	114	233	40	8.89	3.80	923	103
$\sigma\pm$			0.74	0.34	126	12.1				1.10	0.50	166	16.4			0.80	0.24	166	10.3
$\sigma_m\pm$			0.16	0.07	26.8	2.6				0.24	0.10	35.4	3.5			0.28	0.09	58.7	3.6

Rat W109 ♀. Control, paired feeding, 16 days. Body wt., 210 gm.

Weight of whole fresh liver	7.020 gm.
Weight of sample of fresh liver	456 mg.
Weight of fixed sample before embedding (W_{air})	305.1 mg.
Weight of same sample in oil (W_{oil})	87.5 mg.
Specific gravity of oil (S)	0.8122
Volume of sample ($V=\frac{W_{air}-W_{oil}}{S}$)	268.0 cu. mm.
Assumed total volume of liver	4.124 cc.
Thickness of section (t) = 6μ; average diameter of nuclei (d) = 7.2μ; number of nuclei (n) counted in calibrated area = 410	
Number of nuclei in 1 cu. mm. ($N=\frac{6.51\ n\times 1000}{t+d}$)	202×10^3
Number of nuclei in whole liver	835×10^6
Hepatic cells/gm. of fresh liver	119×10^6
Hepatic cells/100 gm. body weight	398×10^6

In addition, a further histological study was made of each specimen in which cell diameters were measured, cells in mitosis and binucleate cells were counted, and the amount of material surrounding the hepatic cells was estimated.

RESULTS AND DISCUSSION

The data on body and liver weights and on the hepatic cell counts are given in table 1 and in figure 1. In 15 pairs the animals were killed after periods of 24 hours to 6 days, in order to determine any sudden bursts or peaks of mitotic activity which

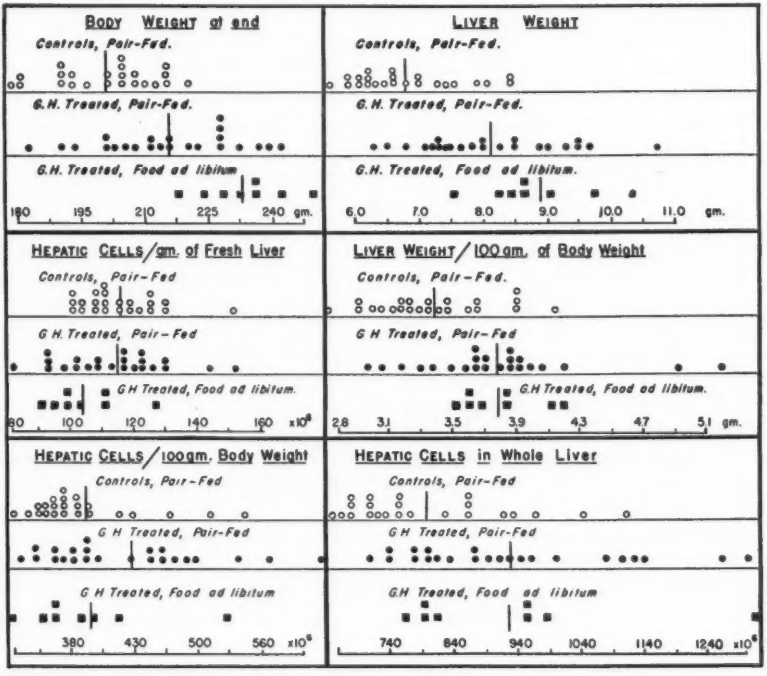
Body Weight of and
Controls, Pair-Fed.
G.H. Treated, Pair-Fed.
G.H. Treated, Food ad libitum
180 195 210 225 240 gm.
Liver Weight
Controls, Pair-Fed.
G.H. Treated, Pair-Fed.
G.H. Treated, Food ad libitum.
6.0 7.0 8.0 9.0 10.0 11.0 gm.
Hepatic Cells/gm. of Fresh Liver
Controls, Pair-Fed
G H Treated, Pair-Fed
G H Treated, Food ad libitum.
80 100 120 140 160 x10⁶
Liver Weight/100 gm. of Body Weight
Controls, Pair-Fed.
G H Treated, Pair-Fed
G.H Treated, Food ad libitum.
2.8 3.1 3.5 3.9 4.3 4.7 5.1 gm.
Hepatic Cells/100 gm. Body Weight
Controls, Pair-Fed
G H Treated, Pair-Fed
G H Treated, Food ad libitum
380 430 500 560 x10⁵
Hepatic Cells in Whole Liver
Controls, Pair-Fed
G H Treated, Pair-Fed
G.H Treated, Food ad libitum
740 840 940 1040 1140 1240 x10⁶

Fig. 1. Data on body and liver weight and hepatic cell counts. Mean values are indicated by a vertical line in each distribution of individual values.

might ensue soon after starting treatment with growth hormone. The means given in table 1 are calculated for the whole series of 23 pairs, although the differences between control and pair-fed treated groups are not great in the experiments of 1 and 2 days' duration. The differences in the means are significant despite the inclusion of these short-period experiments.

Gains in body and liver weights. Every treated rat in the paired series gained more in body weight than its control mate, although its food consumption was restricted to exactly the same amount. For the whole series the 23 treated animals made an average gain of 17.8 gm. (8.7%) each more than the controls. The average liver weight in the treated group was 1.36 ± 0.29 gm. (20.1%) greater than in the controls ($P^2 < 0.001$). The average percentage increase in liver weight as a result of treatment was thus more than twice as great as the increase in total body weight. Consequently, the ratio of liver weight to body weight was significantly higher in the treated rats (difference $= 0.41 \pm 0.12\%$; $P < 0.001$).

Increase in hepatic cells. The larger liver mass, on the average, in the pair-fed treated rats was characterized by a significantly greater total number of hepatic cells (difference $= 138 \pm 44 \times 10^6$; $P < 0.001$). The average number of hepatic cells per gram of fresh liver, however, was essentially identical in the two groups. Likewise the gain in number of hepatic cells per gram of increase in liver weight in the treated group did not differ significantly from the number of cells per gram of liver in the controls.

Mitosis in liver cells. We were unable to detect any significant difference in the rate of mitosis in control and treated rats. Even in the 8 pairs sacrificed within 24 to 60 hours after starting treatment no bursts of mitotic activity were found. There was also no indication that amitotic division was occurring. Brues and Marble (7) reported that in the livers of normal rats only an occasional mitosis can be found, never more than 1 in 10,000 or 20,000 hepatic cells. After removal of about 68% of the liver they found that the residual mass regenerated rapidly, increasing 100% in weight within the first 48 hours. During this time the maximal rate of mitosis occurred averaging 2.13%, or at least 200 to 400 times the resting rate. The stimulus to liver growth as a result of treatment with A P growth hormone is obviously much milder, and possibly different qualitatively, than that responsible for the regeneration of liver fragments. In our treated rats the average total increase in liver weight over the controls was 20% and the average increase in number of hepatic cells was 138×10^6. An increase of only 4 to 8 times in the rate of mitosis in the livers of the treated rats over the normal rate in controls would account for all of this gain in hepatic cell number, but would be extremely difficult to demonstrate by counts of mitotic figures. The use of colchicine might have made it possible to demonstrate a difference, but we did not use it because of its complicating toxic effects.

Histological study. The cells of the liver other than hepatic cells were not counted, but comparison of liver sections from control and treated rats revealed no differences in the microscopic architecture, and it was impossible from observation to determine whether a given section came from a treated or a control rat. The relative proportions of the structural units (liver cords, blood vessels, portal units and stroma) were the same in both groups. We conclude that under the influence of growth hormone the liver responds with a true hyperplasia, with all of the components participating equally.

With the assumption that the nuclei of hepatic cells are ellipsoidal or spherical

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[2] From Fisher, R. A., Statistical Methods for Research Workers. Oliver & Boyd, London, 1930.

in shape, estimations of their relative average size in the three groups of rats were made by measuring the two diameters. The means were: controls, $7.12 \pm 0.06\mu$; treated rats, paired feeding, $7.20 \pm 0.08\mu$; treated rats with food ad libitum, $7.50 \pm 0.04\mu$. This indicates that the hepatic cell nuclei in the treated rats unrestricted as to food consumption were significantly larger than those of the controls (difference $= 0.38 \pm 0.07\mu$; $P < 0.001$). Estimations of whole hepatic cell size from measurements of cell diameters were not considered reliable.

Gains in ad libitum-fed group. In the group of treated rats allowed food ad libitum the gains both in body weight and liver weight were greater than in their treated mates restricted in food consumption. Again, the gain in liver weight (31.5%) was relatively greater than the gain in total body weight (20%), and the liver weight: body weight ratio was significantly higher than in controls (difference $= 0.44 \pm 0.12\%$). The absolute number of hepatic cells was, on the average, practically the same as that in the pair-fed treated group. As a consequence of the greater liver weight, the number of cells per gram of fresh liver ($103 \pm 3.6 \times 10^6$) was lower than in the controls (difference $= 13 \pm 4.4 \times 10^6$; $P < 0.01$), or than in the treated pair-fed group.

The simplest explanation would seem to be that under the influence of growth hormone the livers of both the pair-fed and ad libitum-fed treated groups were stimulated to the same degree with respect to hepatic cell reproduction, but that the plethora of nutrients available in the ad libitum-fed group allowed the growth of larger hepatic cells. It is interesting in this connection that Brues, Drury and Brues (6) found that the rate of regeneration of the residual liver mass after partial hepatectomy was much slower in fasted than in fed rats, but that the rate of increase in the number of hepatic cells was approximately the same in fasted and fed animals.

Nitrogen content. Brues, Drury and Brues (6) found that in the regenerating liver after partial hepatectomy the material added during the first two days contained considerably less nitrogen then resting liver tissue. This was not the case with the material added to the liver in the present experiments with growth hormone. The livers of the 8 control rats in the experiments lasting 24 to 60 hours (table 1) contained an average of 33.0 ± 0.63 mg. of nitrogen per gram of fresh liver; the livers of their 8 growth-hormone treated mates contained an average of 33.6 ± 0.71 mg. of nitrogen per gram of fresh liver.

Chemical analyses were not made of the animals listed in table 1 that were kept for 16 days. Data on nitrogen content are available from another series, however, in which 16 trios were treated in exactly the same way. These are summarized in table 2.

TABLE 2. EFFECT OF GROWTH HORMONE TREATMENT ON LIVER WEIGHT AND NITROGEN CONTENT
VALUES ARE MEANS AND σ_m

Group	Initial body wt.	Final body wt.	Liver wt.	Ratio L.W./B.W.	Liver nitrogen
	gm.	*gm.*	*gm.*	%	*mg./gm.*[1]
16 normal controls. Paired feeding	210±4.1	211±4.0	7.08±0.16	3.35±0.04	33.5±0.40
16 G. H. treated. Paired feeding	208±4.1	230±4.4	8.90±0.28	3.87±0.08	33.4±0.41
16 G. H. treated. Food ad libitum.	208±4.0	240±3.8	9.45±0.22	3.94±0.05	33.0±0.30

[1] Mg. of nitrogen per gram of fresh liver.

In this series also the liver weights and ratios of liver to body weight were significantly greater in the treated rats than in their controls, but there were no essential differences in the nitrogen content of the livers.

SUMMARY

Estimations were made of the number of hepatic cells in the livers of 23 normal control rats, 23 litter mates treated with anterior pituitary growth extract for 1 to 16 days and kept on a paired-feeding regimen, and 8 litter mates treated with growth hormone but allowed food ad libitum.

The average liver weight, the ratio of liver weight to body weight and the total number of hepatic cells were significantly greater in the treated animals than in the controls. The number of hepatic cells per gram of fresh liver was essentially the same in the two groups.

Histological study indicated that there were no differences between the two groups in the proportions of the structural elements of the liver other than hepatic cells.

In ad libitum-fed treated rats the increase in number of hepatic cells was the same as in the treated mates with restricted food consumption, but the increase in mass of the liver was greater and the average diameter of hepatic cell nuclei was greater. The nitrogen content per gram of liver was essentially the same in all groups.

It is concluded that under the influence of the growth hormone the liver responds with a true hyperplasia, with all of the structural components participating equally.

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EFFECT OF A NORMAL MALE URINE EXTRACT ON IMMATURE FEMALE CATS

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IT HAS BEEN SUGGESTED that an extract of normal male urine contains a gonadotropic substance comparable to the anterior pituitary-like follicle stimulating hormone in menopause urine (1–4). Follicle stimulation and uterine weight increase in immature rats and mice has been produced with this extract (5, 6, 7). In these cases, the action of this material was comparable to the action of menopause or castrate urine extract. Normal male urine extract has been shown to be capable of inducing all the manifestations of estrus in the dog as the result of pronounced follicle stimulation (8). Wells and Overholser (9) demonstrated that this hormone could stimulate sperm formation in hypophysectomized mature and immature ground squirrels. Other investigators have reported only slight gonadotropic activity with normal male urine (10, 11).

In this investigation, the manner and capability of the immature cat ovary to respond to an extract of normal male urine was tested and the results compared to those previously obtained with menopause urine extract (12).

EXPERIMENTAL METHODS

For these experiments, 23 immature female cats of known age were employed. Body weights of these animals ranged from 340 gm. to 1400 gm., but most of the animals weighed less than a kilogram. Eighteen animals ranging in age from 5 to 13 weeks were tested with an extract of normal male urine. In addition one cat served as a litter-mate control in one group and control data on 4 other animals was available. When 2 or 3 female cats from the same litter were available, the animals were given identical hormonal treatment to permit a study of the individual variation among litter mates. This was possible with all but 2 animals.

In all cases, sections of the ovaries, uteri and vaginae were prepared. The cats were either killed the day following cessation of treatment or the animal was operated, one ovary and a portion of the corresponding uterus removed, and the animal killed 5 to 10 days later.

Normal male urine extract, prospermin,[2] was dissolved in distilled water and injected subcutaneously. A solution of this material was made up for 2 days' injections.

RESULTS

Control animals. Immature female cats exhibited virtually the same condition despite a variation in age of from 6 to 12 weeks. The vaginal smears consisted of epi-

[1] A portion of this investigation was carried out in the Department of Biology, University of Pittsburgh, Pittsburgh, Pa.

[2] Normal male urine extract, "prospermin," was furnished through the kindness of Dr. J. A. Morrell of E. R. Squibb & Sons.

thelial cells and a few leucocytes. The uterus was small and the endometrium showed only slight, if any, glandular development (fig. 1). The ovaries contained only small vesicular follicles (fig. 4).

Experimental animals. The uterine condition of the experimental animals closely paralleled the state of the ovary. A proestrous or estrous endometrium and hypertrophy of the myometrium occurred in correspondence with the degree of follicle stimulation (fig. 2). If a period of 5 to 9 days was allowed to elapse after the last injection, lutein tissue was observed in the ovary and the endometrium was in a pseudopregnant state (fig. 3). No regression of the endometrium was observed.

In one of 2 cats, when hormone administration was extended over 21 days and the ovaries contained follicles and corpora lutea, a pseudopregnant endometrium was observed. This pseudopregnant state was also seen in an animal treated for a 36-day period.

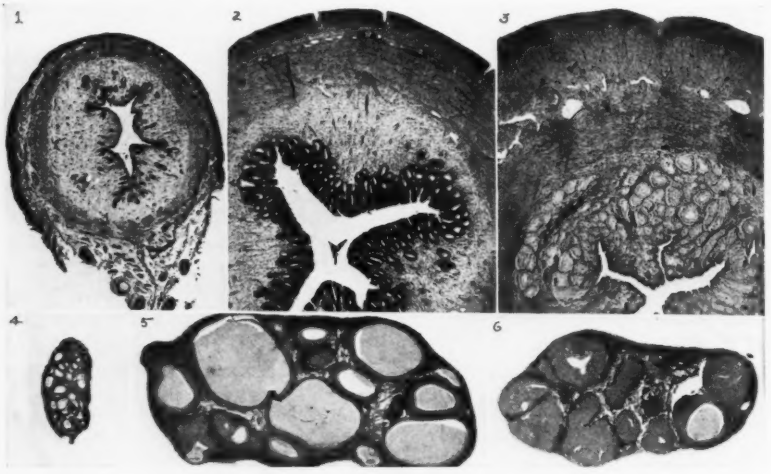

Fig. 1. NORMAL UTERUS OF AN IMMATURE CAT. ×38. Fig. 2. ESTROUS UTERUS AS RESULT OF INJECTION OF NORMAL MALE URINE EXTRACT. ×19. Fig. 3. UTERUS OF A CAT 5 DAYS AFTER LAST INJECTION. PSEUDOPREGNANT TYPE. ×19. Fig. 4. NORMAL OVARY OF AN IMMATURE CAT. ×3.1. Fig. 5. OVARY SHOWING PRONOUNCED FOLLICLE STIMULATING EFFECT OF NORMAL MALE URINE EXTRACT. EVIDENCE OF THICKENED GRANULOSA. ×3.1. Fig. 6. OVARY SHOWING ATRETIC CORPORA LUTEA RESULTING FROM PROLONGED TREATMENT WITH NORMAL MALE URINE EXTRACT. ×3.1.

Eleven immature female cats were given 10 daily injections of 20 R.U. of normal male urine extract in 11 days. Two of a litter of 3 cats, 5 weeks old, failed to respond. However, the third cat showed the stimulation of one follicle to large size in the right ovary whereas the left ovary was typically immature.

Four cats, 6 weeks old, were treated similarly and a response was observed in all cases. Three of the cats were litter mates, but only one exhibited follicle stimulation in both ovaries. In 2 animals, 2 follicles were stimulated to medium size in the right ovary whereas no reaction was observed in the left ovary. There was no appreciable difference in the weights of the left and right ovaries. Several follicles were stimulated to good size in the absence of corpora lutea in both ovaries of the third cat. Normal ova were present. A marked stimulation of a single follicle in each ovary was observed in the fourth cat of this age group. Corpora lutea were absent but the ova in

the large follicles appeared abnormal and were probably becoming atretic. Several smaller follicles showed definite signs of atresia.

Two litters of 2 cats each, all of which were 11 weeks old, responded to this extract in a comparable manner. One cat from each litter showed a moderate response in the stimulation of follicles and ovarian weight increase. A marked response was observed in the other 2 cats. *Cat 60* was operated the day following cessation of treatment with the removal of the left ovary and a section of uterus. This ovary was significantly increased in weight, being 874 mg., in comparison to an ovarian weight range of 30 to 100 mg. for a single ovary from control animals of varied ages. This ovary contained several follicles of very large size, but the ova in several instances appeared to be abnormal, possibly due to excessive stimulation. The granulosa also was abnormal in some cases, but corpora lutea were absent (fig. 5). This cat was killed 5 days later. The right ovary, with the exception of one large follicle, consisted entirely of varied sized corpora lutea atretica.

The other cat (animal 66) was operated on the 12th day after the initial injection and the left ovary weighed 342 mg. This ovary contained many normal follicles stimulated to good size in the absence of corpora lutea. However, the stimulation was less pronounced than in *cat 60*. Nine days later, the animal was killed and the right ovary was found to consist entirely of many small corpora lutea atretica. The size of the corpora conformed to the size of the follicles observed in the left ovary and did not show signs of degeneration.

Additional cats were subjected to varied treatment. Two 7-week-old cats received 8 daily injections of 20 R.U. in 9 days. Increase in the number of follicles was evident, but large follicles were not present.

Two cats, 10 weeks old, were injected every third day with 30 R.U. and were killed on the 19th day after receiving a total of 180 R.U. Although these animals were litter mates, only one responded to treatment. The ovarian reaction was marked, but under this treatment lutein tissue predominated although a few large follicles were present. A slightly higher dose administered in 11 days produced no corpora lutea.

A total of 260 R.U. in 18 injections was administered to a 13-week-old cat over a period of 36 days. Both ovaries were greatly stimulated, but atretic follicles and corpora lutea comprised all of the ovarian tissue (fig. 6). Two other cats of the same age received 340 R.U. in 15 injections in 21 days. In both animals, the ovaries were greatly enlarged and consisted of follicles with thickened granulosa, cystic follicles and corpora lutea atretica. The lutein tissue was vacuolated in some cases and appeared to be degenerate. Lutein tissue that was observed after injections were discontinued in previous experiments was entirely normal in appearance.

DISCUSSION

Normal male urine extract, prospermin, was found to contain a gonadotropic substance capable of producing follicle stimulation in the immature female cat. Animals treated with 20 R.U. daily for 10 days showed only follicle stimulation. In many instances, the follicles were of ovulating size. Normal ova were generally present. This ovarian reaction was quite comparable to the stimulation obtained with menopause urine extract (12). However, abnormal ova and signs of degeneration were observed in some cases of apparently excessive stimulation. If the extract was administered over a longer period of time, both follicles and corpora lutea atretica were present. Two cats had ovaries which were predominantly lutein in nature. Further evidence of ovarian stimulation was indicated by as much as an 8-fold increase in

ovarian weight. A single ovary from one cat weighed 874 mg. as compared to the control range of 30 to 100 mg.

It was previously observed with menopause urine extract that the right ovary responded in 3 cats in which the left ovary remained immature, but the reverse condition was not observed (12). In these experiments, 3 more cats fell into the category in which the right ovary responded whereas the left ovary had not reacted. The other animals either failed to respond or else both ovaries were stimulated.

A considerable variability in the degree of response to the same total dose was, obtained with litter-mate animals. In one litter, only one of 3 cats responded, whereas in other litters each animal responded more or less markedly. Thus, it appears that the age of the animal was not the factor determining the response, but rather the capability of the individual ovary to react at the time of treatment.

A proestrous or estrous endometrial development corresponded to the degree of follicle stimulation. Presence of lutein tissue in the ovary resulted in a pseudopregnant condition. No regression from this state was observed 9 days after the last injection.

SUMMARY

Fifteen of 18 immature female cats, 5 to 13 weeks of age, responded to an extract of normal male urine, prospermin. Ovarian weight increased as much as 8-fold over controls. After 11 days treatment, only follicle stimulation was observed but extended treatment resulted in the formation of corpora lutea atretica, although the total dose was fairly comparable in some cases. In most cats, both ovaries either did or did not respond, but in 3 animals only the right ovary responded. Furthermore, considerable variation of the degree of stimulation resulted among litter-mate cats that had received the same hormonal treatment. The capability of the individual ovary to react at the time of treatment appeared to determine the response rather than the age of the cat. An estrous or pseudopregnant endometrium developed in association with the state of the ovary.

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OBSERVATIONS ON SPERMATOGENESIS IN RATS

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THE DISCOVERY by Walsh, Cuyler and McCullagh (1) that androgens will maintain spermatogenesis in hypophysectomized rats has been adequately confirmed (2–5). It has also been shown that male hormones will maintain the seminiferous tubules in hypophysectomized mice (6) and in hypophysectomized rabbits (7). No maintenance effects have been observed in hypophysectomized guinea pigs (8) or in hypophysectomized monkeys (9) injected with androgens.

The maintenance of spermatogenesis by androgens in hypophysectomized animals has been interpreted as an indirect and a direct response to the male hormones. Cutuly et al. (3, 5) suggested that the tubular maintenance was not necessarily due to a direct action of male hormone on the germinal cells. These investigators pointed out the possibility that spermatogenesis might occur in hypophysectomized animals injected with androgens simply because the androgens stimulate the scrotum, and that maintenance of this organ would provide the gametes with a favorable environment in which they could develop independently of any known direct hormonal influence. Other investigators have disagreed with the view postulated by Cutuly et al. and have favored the idea that male hormones exert a direct stimulative effect upon spermatogenesis (4, 10).

It must be admitted that there is still no conclusive evidence to support either the direct or the indirect action theory of spermatogenic maintenance. Hamilton and Leonard (11) and Leonard and Hamilton (12) have reported experiments which they feel invalidate the indirect action theory, but their conclusions do not seem to rest on sound reasoning. Thus, because androgens were found to hasten the degeneration of the germinal elements in the testes of cryptorchid rats, they stated (11) that male hormones act directly on the seminiferous tubules. Whatever the reason for the apparent acceleration of the germinal degeneration noted by these authors, the implication, on the basis of such results, that androgens are gametokinetic seems illogical. The rationale of the experiments reported by them (12) is not clear. They anchored testes of hypophysectomized rats in the scrotum and compared the rate of degeneration in such anchored testes with that in testes which were left free to ascend, presumably away from the favorable environment of the scrotum. No difference in the rate of degeneration was noted whether the testes were sutured in the scrotum or left free. The anchoring of the testes in the scrota of hypophysectomized rats seems rather pointless. It has been shown that scrotal function depends upon the presence of testicular hormone in the organism (3, 13, 14). Since there is no reason to believe that testicular hormone does not disappear from the body almost as rapidly after hypophysectomy as it does after castration, it would follow from the studies of Moore et al. (15) that scrotal function is impaired within a few days after ablation of the pituitary. Hence, there was little reason for Leonard and Hamilton to expect a favorable action to be exerted by the scrotum after hypophysectomy.

Heretofore studies on the effects of androgenic substances on the testicles of hypophysectomized rats have been made on adult or immature (16) animals. It seemed of importance to determine what effects would occur in young animals, as well as in immature and adult ones. This report presents the results of such a study.

[1] Part of the work presented in this report was done at the Cleveland Clinic, Cleveland, Ohio.

MATERIAL AND METHODS

In all instances treatment consisted of subcutaneous injections of 3 mg. of androgenic substance[2] daily for 23 or 24 days, autopsy being performed on the day after the final injection. Injections in hypophysectomized rats were begun one day after operation. The large daily dose of 3 mg. was selected because it seemed fairly certain that the possibility of under-dosage would thus be eliminated, and no evidence has thus far been adduced which indicates that heavy doses of androgen have deleterious effects on spermatogenesis in hypophysectomized rats.

Data in table 1 are given for 2 series of rats. The first series comprises immature rats aged 29 or 34 days. The second series is made up of rats of ages ranging from 47 to 177 days.

TABLE 1. AVERAGE BODY WEIGHTS AND WEIGHTS OF TESTES OF NORMAL AND OF HYPOPHYSECTOMIZED RATS SHOWING EFFECTS OF INJECTIONS OF 3 MG. OF ANDROGEN DAILY FOR 23 OR 24 DAYS, WITH AUTOPSY ON THE DAY AFTER THE LAST INJECTION

Series	Age at start	No. of rats	Animal group and treatment	Body weight		Weight of testes
				Initial	Autopsy	
	days			*gm.*	*gm.*	*gm.*
		2	Hypophysectomy—TP	52	55	0.31
	29	1	Initial control	—	55	0.43
		1	Normal control	44	142	1.38
I	29	3	Normal—TP	55	135	1.55
		1	Normal control	50	130	1.43
		2	Hypophysectomy—TP	105	106	0.69
	34	1	Initial control	—	87	0.59
		1	Normal control	89	182	2.47
		3	Hypophysectomy—TP	131	130	2.13
	47– 53	4	Hypophysectomy—DAC	130	129	1.57
		2	Normal control	142	190	2.51
	63– 65	4	Hypophysectomy—TP	173	146	2.21
II		4	Hypophysectomy—DAC	192	172	2.11
		4	Hypophysectomy—TP	195	174	2.24
	75– 78	4	Hypophysectomy—DAC	196	182	2.39
		2	Normal control	197	230	3.00
		3	Hypophysectomy—TP	257	210	2.25
	106–108	3	Hypophysectomy—DAC	261	207	2.80
		1	Normal control	226	258	3.29
		4	Hypophysectomy—TP	294	266	3.05
	131–177	3	Hypophysectomy—DAC	314	269	2.90
		2	Normal control	293	325	3.60

TP=Testosterone Propionate DAC=Dehydroandrosterone Acetate

Series I. Of the 4 animals in the first litter 29 days of age 2 were hypophysectomized and treated with testosterone propionate, one was killed at the start of the experiment (initial control), and one served as a normal control. In the second litter, 29 days of age, 3 normal rats were treated with testosterone propionate and one served as a normal control. The third litter of rats was 34 days old when the experiment was begun. Two of the animals were hypophysectomized and injected with

[2] We are indebted to Dr. Erwin Schwenk, Schering Corp., Bloomfield, N. J., for the androgens used in this work.

testosterone propionate, one was autopsied at the beginning of the experiment (initial control), and one was used as a normal control.

Series II. The members of litters ranging in age from 47 to 177 days were so separated that litter mates usually were distributed among 3 groups. One group was used as a normal control, and the other 2 groups were hypophysectomized and treated either with testosterone propionate or with dehydroandrosterone acetate.

RESULTS

Series I. Daily injections of 3 mg. of testosterone propionate for 24 days produced no gross or microscopic stimulation of the testes of rats hypophysectomized at 298 days of age. On the contrary the tubular elements exhibited degeneration when compared with those in the initial control of 29 days. In sharp contrast to the effect observed in the hypophysectomized rats the testicular tubules in the 3 normal animals injected with testosterone propionate were indistinguishable from those in the normal control. The interstitial cells in the testes of these injected normal animals, however, were profoundly atrophied. The testes of rats hypophysectomized at 34 days of age and treated thereafter with testosterone propionate did not increase significantly in weight over those of the initial control, but histologically there was a marked distinction. Some of the tubules in the testes of the treated hypophysectomized rats contained what seemed to be structurally normal spermatozoa, whereas gametogenesis in the initial 34-day-old control had not advanced beyond the second spermatocyte stage. Despite the presence of spermatozoa in the testes of the androgen-injected hypophysectomized rats, the tubules were far from normal. Tubules containing spermatozoa appeared decidedly smaller than normal and their epithelial investments gave the impression of being loose and scanty. Other tubules which did not contain spermatozoa were markedly shrunken. This condition resembled, in general, that which was observed in 29 and 30-day-old hypophysectomized rats the testes of which developed spermatozoa following the administration of testosterone propionate (16).

Series II. Young and adult hypophysectomized rats were injected daily for 24 days with 3 mg. of testosterone propionate or of dehydroandrosterone acetate. Comparison of the testicular weights (table 1) of the treated animals and those of the normal controls reveals no striking differences except in the case of the 47 to 53-day-old hypophysectomized group to which dehydroandrosterone acetate was administered. The average weight of the testes in this group was 1.57 gm. as compared with the normal control value of 2.47 gm. This weight maintenance of 64% is interesting, for in 2 adult hypophysectomized groups (5) it was found that dehydroandrosterone acetate effected a testicular weight maintenance of 84%. With this exception, testicular growth and development in all of the injected groups compared very favorably with that which occurred in the normal controls. Histologically, too, the seminiferous tubules of the testes in the injected rats did not differ appreciably from those in normal animals. Though, as has been mentioned previously (3, 5), occasional groups of atrophic tubules were noticed in sections of testes from the hypophysectomized animals receiving androgens. In all probability these groups of degenerated tubules and the atrophic interstitial cells were chiefly responsible for the testicular weight differences between treated and normal animals. It should be noted that testicular weights in the treated groups were in no instance as great as their respective controls, and that the absolute weights of maintained testes were greater in the older and heavier animals.

DISCUSSION

The results emphasize the importance of age in the evaluation of studies on spermatogenesis. It will be of interest to know to what extent androgens will maintain or induce spermatogenesis in rats hypophysectomized between the ages of 34 and 47 days. Since the present results indicate that age will play an important part in this relatively short range of 34 to 47 days, an extensive investigation of the testicular responses in this age group is planned. The data as they now stand suggest that within certain limits the degree of testicular maintenance by androgens in hypophysectomized rats increases with the age of the animals. Further, the present data, together with those published previously (16), indicate that the possibility of inducing spermatogenesis by androgens in immature hypophysectomized rats becomes increasingly difficult with decrease in age of the animal. In this respect, as well as in their ability to reestablish spermatogenesis in hypophysectomized animals (2–5), androgens differ from pituitary gonadotropins. These observations indicate that gametic maturation is not independent of hypophyseal stimulation. In the light of the knowledge available at present it is possible tentatively to suggest the following interpretation of spermatogenesis.

It is known (17) that spermatogenesis may progress through the primary spermatocyte stage in the absence of the pituitary (and therefore in the absence of testicular hormone as well). It is also known (16) that mature spermatozoön formation may be induced by androgens in immature hypophysectomized rats the testicular tubules of which probably contained only spermatogonia, primary and secondary spermatocytes at the beginning of the injection period. By elimination it would appear that the critical period in spermatogenesis which is hormonally controlled by the anterior pituitary is the stage between primary and secondary spermatocyte, or the stage of reduction division. Stages of gametogenesis succeeding the reduction division must certainly be independent of hypophyseal stimulation, since it has been shown that sperm formation can be induced by androgens in rats hypophysectomized when their testes contained at best secondary spermatocytes (16). Whether the post-meiotic stages of spermatogenesis are directly or only indirectly dependent upon male hormone can not yet be satisfactorily answered. Additional studies which may help to clarify this problem are in progress.

SUMMARY

Immature, young and adult hypophysectomized and immature normal rats were injected with 3 mg. of testosterone propionate or of dehydroandrosterone acetate daily for 23 or 24 days.

Spermatogenesis was induced in 34-day-old hypophysectomized rats, but not in 29-day-old hypophysectomized animals injected with testosterone propionate. In the testes of normal 29-day-old rats to which testosterone propionate was administered spermatogenesis progressed normally, but the interstitial cells were atrophied.

The testes of young or adult rats hypophysectomized between the ages of 47 to 177 days and injected with either dehydroandrosterone acetate or testosterone propionate were in most instances well maintained near the weight level of the respective normal controls. Maintenance in older hypophysectomized animals was therefore at a higher level than in younger ones.

A theory to explain the various phases of spermatogenesis in the light of existing knowledge is tentatively suggested. Spermatogenesis is divided into three phases: a pre-meiotic phase, a reduction division phase, and a post-meiotic phase. The first seems to occur independently of any known hormonal stimulation, the second

seems to be controlled by hypophyseal gonadotropin, and the third is either independent of any recognized hormonal stimulation or else it progresses under the influence of testicular hormone.

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THE EFFECT OF MALE SEX HORMONE ON THE DEVELOPING OVARIES OF YOUNG FOWLS[1]

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STUDIES on the interaction of sex hormones with the pituitary are establishing still more firmly the knowledge that a relationship does exist and that sex hormones from one sex are effective in animals of the same or even the other sex. It is well established that the injection of estrin depresses the ovary (1–4) and that male sex hormone depresses the testis (4, 5). Estrin also appears to be able to depress the testis (2), and male sex hormone the ovaries (6, 7, 8). There is considerable evidence that depression of the gonads is due to suppression of the pituitary and not to the effect of the injected substance directly. Further evidence that injected sex hormones suppress the pituitary comes from clinical reports that either estrin or male sex hormone injected into patients at the time of natural or artificial menopause may correct or relieve menopause symptoms in human females (9–12). The purpose of these experiments was to determine still further the effect of male sex hormone, testosterone propionate, on ovaries.

PROCEDURE

The animals used in this series of experiments were 87 female single-comb White Leghorn fowls. The material injected was male sex hormone, testosterone propionate,[2] containing 5 mg. active substance per 1 cc. of fluid. The dosage in groups I–IV was 0.1 cc. of fluid or 0.5 mg. of active substance and each bird received 5 injections. In group V the dosage was 0.5 cc. of fluid and each bird received 10 injections. Injections were made intermuscularly on alternate days in all birds.

When the birds were killed the body weight was taken, the combs were measured (length and height), and the ovaries were removed and weighed in stoppered bottles on an analytical balance. The ovaries were subsequently sectioned and stained for study. To evaluate the degree of development of the ovaries, representative sections were selected and the total follicles were counted (400 to 700 usually were counted in a single section). By means of an ocular micrometer those follicles 50 micra and 75 micra or over in diameter, respectively, were counted. The results of these counts appear in the table as percentages of the total number of follicles.

RESULTS

For the larger number of animals it was intended to give a relatively small dosage of male sex hormone, but large enough to cause marked development of the combs and wattles. In group V, the head furnishings were larger than those of males of the same age and some of the females were crowing. In groups I to IV, the combs were larger among those that received the male sex hormone as recorded in table 1. Since

[1] Contribution No. 209, Department of Zoology.

[2] The writers are indebted to the Schering Corporation and to Ciba Pharmaceutical Products, Inc, for liberal contributions of the male sex hormone used in this series of experiments.

there was no significant difference between the body weights of the injected birds and their controls, these results were omitted.

In group I, 10 chicks, 2 days old, were given their first injection; and after 5 injections on alternate days, they were killed when 12 days old. The average weight of the control ovaries (10 birds) was greater than the experimentals, but by only 0.00340 gm. The average numbers of follicles 50μ and 75μ in diameter were slightly greater in the controls than in those that received the male sex hormone (Table, group I).

TABLE 1

Group	Av. % follicles over 50μ	Av. % follicles over 75μ	Av. comb size at start	Av. comb size at end	Av. ovary wt.
			mm.	*mm.*	*gm.*
I					
Exper.	9.97	1.54	10.08×2.50	21.68×11.0	.01320
Control	11.48	1.73	10.62×2.77	14.17×4.08	.01660
(diff.)	1.51	0.19		7.51×6.92	.00340
II					
Exper.	12.46	2.98	10.08×2.50	23.60×9.60	.0370
Control	16.98	3.31	10.62×2.77	14.50×4.20	.0370
(diff.)	4.52	0.33		9.10×5.40	.00
III					
Exper.	14.44	3.32	10.08×2.50	24.00×10.4	.04121
Control	26.25	9.35	10.62×2.77	18.00×6.2	.04872
(diff.)	11.81	6.03		6.00×4.2	.00751
IV					
Exper.	12.40	2.42	10.50×2.60	26.60×13.2	.0313
Control	16.98	3.31	10.62×2.77	14.50×4.2	.0370
(diff.)	4.58	0.89		12.10×9.0	.0067

In group II, conditions were similar to those in group I except that the birds were killed when 17 days old instead of 12 days. The average ovary weights in the 2 groups were the same. The follicle development, as in group I, was greater in the control group and to a slightly greater degree (Table, group II).

Group III was conducted in the same way as group I except that the birds were killed on the 17th day after the last injection, when they were 27 days old. This group showed the greatest difference of all between the injected birds and the controls, except in comb size (Table, group III).

Group IV was different from the others in that they received their first injection when 7 days of age. They were killed on the second day after the last injection when 17 days of age. Consistent with the other groups, the control birds showed slightly greater development of the ovaries than those that received male hormone. The difference in the comb size was greatest in this group (Table, group IV).

Group V consisted of 4 female fowls and 3 controls. They were 134 days old at their first injections of 0.5 cc. of male hormone on alternate days. They were killed on the second day after the tenth injection. Follicle counts were not made, but the average weight of the control ovaries was 11% greater than those from the injected birds.

It is recognized that these groups were not large, but it is believed to be signifi-

cant that in all cases those that received the injections showed a lesser degree of follicle development than did their controls. In all but one group, those that received the injections, had ovaries lighter in weight than their controls. In group II the weights were the same. There is indication that even small doses of male sex hormone suppresses ovarian development in young female fowls.

SUMMARY

Young female chicks that received small doses of male sex hormone (a total of 2.5 mg. testosterone propionate) had ovaries slightly smaller than their controls (in one group they were the same as their controls), and follicular development was somewhat less than in the controls.

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PRECOCIOUS COPULATORY ACTIVITY INDUCED IN MALE RATS BY SUBCUTANEOUS INJECTIONS OF TESTOSTERONE PROPIONATE[1]

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Gonadotropic and gonadal hormones induce many of the somatic and physiological signs of pubertas praecox in immature rats if administered repeatedly in appropriate doses (1, 2). Whether these somatic changes are accompanied by psychosexual changes, however, cannot be definitely concluded from the published studies available to the author. Reports on the masculinization of young chicks suggest that the somatic and psychosexual changes may, to a marked extent, go hand in hand. Domm and v. Dyke (3) reported that male chicks crowed at the age of 9 days, following 6 daily injections of a gonadotropic hormone (hebin), and that treading appeared when the chicks were 13 days old, following 10 daily injections of hebin. Hamilton (4) caused male chicks to crow at the age of 10 days, by injections of testosterone propionate from the second day after hatching. These chicks struck with their feet as though attempting to use spurs (which they did not have) and pecked belligerently at intruding objects. However, treading or other evidence of copulatory aggressiveness was not mentioned by Hamilton in this report or in the report by Breneman (5) who induced crowing in male chicks when they were only 5 days old by daily injections of dihydroandrosterone benzoate. Absence of this element of masculinization suggested to the author the desirability of further experimental studies of the behavioral aspects of pubertas praecox in laboratory animals.

In the present study the chief objectives were to determine whether precocious copulatory behavior could be induced in immature male rats by subcutaneous injections of testosterone propionate, and to compare this activity with that normally appearing at a later time in untreated males.

Animals. The young rats were reared in our own laboratory. Their diet and also that of their parents consisted of the Steenbock mixture (6) supplemented by lettuce one day per week. They were weaned when 25 days of age. No special selection of litters or of individuals was made when forming the control and experimental groups. Because of this, body weights at weaning, sizes of litters and numbers of males per litter are variable. The groups on the whole, however, appear to be representative of the colony with which we have worked on problems of sex during the past decade and a half at Stanford University.

Injections. A close approximation of 0.62 mg. of testosterone propionate dissolved in one-eighth of one cc. of sesame oil was injected beneath the loose skin over the shoulders or at the flank of each experimental animal daily from the beginning of in-

[1] This study was financed by the Committee for Research on Sex Problems, National Research Council. My research assistant for the observational part of the study was Mr. Max W. Lund, graduate student in psychology, to whom I am greatly indebted for painstaking effort to adhere to a standard procedure in testing the males.

jections to the day on which the first copulatory activity was recorded. Animals with numbers falling between 1 and 18, inclusive, received their first injections when 25 or 26 days of age; those with numbers between 19 and 51 received theirs when they were 22 days old. Some of the injected substance followed the needle as it was withdrawn or was pressed out by the animal later on as it moved about the cage. Although the small loss of hormone probably was of no consequence to the individual injected, since it would be effective even on the surface of the skin (7), the point is mentioned because the litter-mate controls were kept with the injected animals in order to equalize, so far as possible, the factors of husbandry and testing of litter mates. Anticipating our test results at this point, it may be said that there is no indication that the controls absorbed sufficient hormone to affect their psychosexual development in a measurable degree. Control injections of pure sesame oil into the normal animals were not made. It seemed unnecessary to subject these young to this type of control as no experiments so far reported have suggested that this substance alone has androgenic effects. Repeated injections of the oil might, however, have a retarding effect on sexual development.

Acceleration of growth rate of the external genitals of injected animals (testes excepted) was apparent almost immediately (cf. 1). Within 24 hours after the first injection there was a change in the redness of the prepuce. On the third day elongation of the penis could be detected. By the fifth day the glans was well developed and could be exposed by the animal itself or by manual retraction of the foreskin. At this time the penis resembled that of an untreated male of the age of 45 to 50 days. Although the scrotum became more pendulous than usual the testes appeared not to be accelerated in growth rate. Whether or not there was actually some retardation, as one might expect in accordance with the study of Moore and Price (8), we did not attempt to ascertain. Erection of the penis was noted many times as the young males made copulatory attempts or sat back on tail and hind feet after copulating.

Tests for copulation. The term copulation is used here to embrace the following elements of the complete copulatory act as it is found in adult males: pursuit of the female; mounting and palpation of the female's sides with forepaws; rapid pelvic movements that terminate by the animal's sitting back on its tail and hind feet and licking the penis. Usually the final, slightly more vigorous pelvic thrust is present but in some instances this is absent. In the latter case the act resembles abortive copulatory acts of adults in which they fail to effect intromission. No instance of the prolonged clasp in which the ejaculatory reflex occurs was recorded.

The tests for copulatory behavior were made between 7:30 and 11:00 P.M. A receptive female was placed with one or two of the young males at a time and left there for a period of from 8 to 10 minutes while the experimenter kept close watch for copulatory behavior. From 2 to 3 tests per evening were given and usually from twenty minutes to three-quarters of an hour elapsed between tests. No distinction between experimental and control animals was made and the cage-mates tested together varied from time to time. The females were relatively small adults weighing about 150 to 175 gm. No female that was wild or pugnacious during the preliminary tests was used lest the young males be intimidated. Even so, it was not possible to prevent occasional instances of biting or mauling of young. This type of behavior, seemingly, cannot be prevented when males that are much smaller than the females are being tested.

RESULTS

The net results of the tests for copulation are presented in figures 1 and 2. The first figure is a frequency distribution of the ages of first copulation of the injected

animals, their litter-mate controls, and a group of non-litter-mate controls similarly tested. The second figure provides a simple basis for comparing the litters as well as the individuals within litters; it also gives the dates of injections and the time of and number of tests for each animal.

Unpublished and published data (9) at our disposal indicate that 35 to 40 days may be regarded as the typical lower range of ages at first copulation in untreated rats which are reared and tested as we have done in the present experiment. The upper age range cannot be fixed so accurately but probably it terminates in the neighborhood of 70 to 80 days (9). For small groups of animals the median age of first copulation usually falls between 47 and 58 days.

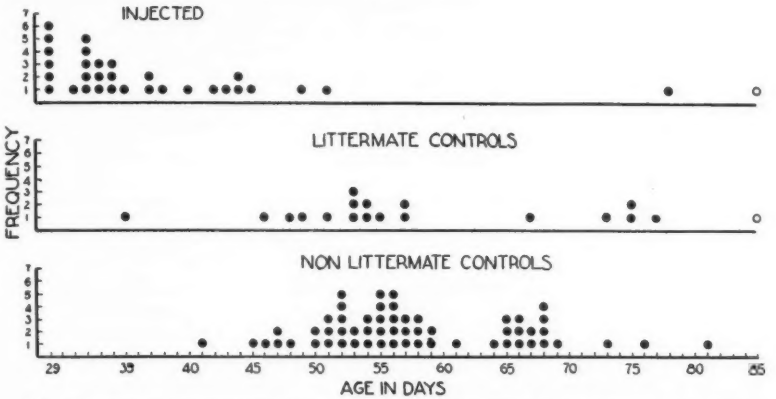

Fig. 1. Frequency distributions of the ages of first copulations of the injected rats, their litter-mate controls, and a group of non-litter-mate controls similarly tested.

Taking the foregoing values as points of reference when considering figure 1, it is clearly apparent that the injected males, as a group, copulated precociously for there is only a small amount of overlapping between them and the controls. For the 9 injected males (*rats* 1 to 18, fig. 2) which received their first treatment when 25 or 26 days of age, the lowest age at first copulation was 32 days and the median age for the 9 animals was 35 days. For the 22 that were injected from their twenty-second day, the earliest age of first copulation is 29 days, and the median age is 32 days. Whether the greater acceleration in the second sub-group is due to earlier injections, to more numerous injections prior to the first tests (cf. fig. 2), or to both of these factors cannot be determined from our present data. Probably earlier testing would have shunted some of the cases now massed at the age of 29 days to lower ages. Massing of cases at the lower end of the range in tests of this kind usually indicates that opportunity for overt sex behavior lagged behind the animal's state of readiness.

That testosterone propionate is not 100% effective in inducing the behavioral signs of pubertas praecox is clear from the instances of relatively late copulation. Animals 11, 21 and 45 (fig. 2) did not at any time display sexual aggressiveness at the early ages when other injected animals began to copulate, and *rat* 45 failed to copulate even within the 85-day period of testing. *Rat* 41 which copulated at the age of 49 days made attempts at the age of 29 and again at 31 days. Copulatory attempts have never been seen in normal animals at such early ages, hence we are probably on safe grounds in crediting him with precocious sexual activation even though the full copulatory pattern was not elicited at an early age. While observing that the injected

hormone did not invariably produce precocious sexual aggressiveness, we must not overlook the possibility that the fault may lie in part in our testing procedures. Some of the receptive females posture before the young males much more aggressively than others and thus provide better lures to elicit sexual behavior. Although it is unlikely that a given male would have a long series of relatively inactive females, the relevancy of this point depends on whether dosage administered by us was inadequate to activate certain males for more than a day or two, and through faulty testing their activation was not detected at that time. The point is deserving of further inquiry.

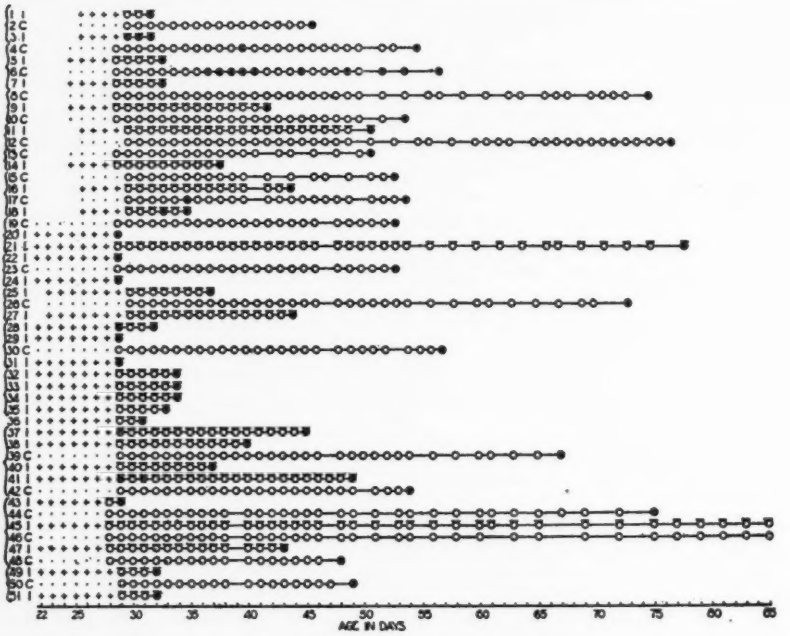

Fig. 2. INDIVIDUAL TEST RECORDS FOR THE INJECTED AND THE LITTER-MATE CONTROLS. Letters I and C refer to injected and control. The animals' ages are given on the abscissa. Circles designate the ages at which tests for copulation were given. Open circles mean that the animals failed to copulate and closed circles (dots) indicate that the males copulated in one or more of the tests given at that age. Plus signs indicate the days on which injections were given prior to the beginning of testing; thereafter a short line that is tangent to the circles indicates this. A cross inside an open circle indicates that the male made copulatory attempts, but failed to complete the act.

With respect to variability of initial copulation in the experimental and the control groups it seems apparent that the greater degree is present in the controls. This statement is based on inspection rather than on computed measures because the varied ages at the time of initial injections of the experimental animals would tend to increase the variability of this group beyond that normally present if all animals had been injected at the same age. The greater variability in the untreated group is explicable if we assume that there is considerable variability in the ages at which an amount of testicular hormone that is adequate to arouse the copulatory impulse normally appears in the blood stream. In the experimental group we should expect less variability because of the synchronous appearance of a relatively large amount of

the male hormone in the blood stream as a result of the subcutaneous injections. Moore and Price (8) have found that the beginning of rapid increase in hormonal secretion by the rat testis is between the ages of 35 and 40 days. They based their statement on the trend of means, however, and did not attempt to deal specifically with individual differences. From marked differences that are apparent in time at which the penis of rats undergoes the puberal growth spurt it would seem that we may safely infer marked differences in the time of great acceleration of hormonal output by the testis. A weakness in these a priori deductions, however, is the fact that we lack precise data on the relative amounts of hormone required to induce copulatory behavior on the one hand and accelerated growth of the various elements of the accessory sexual organs on the other.

Considering the data of this study in their entirety the most striking result is the change in the developmental schedule of the injected rats, in the psychosexual sphere. The awakening of the copulatory impulse has been predated, on the average, by approximately 20 days. The precocity was induced without alteration of the pattern of the copulatory response as it normally appears in untreated young males. The ultimate fate of the precociously awakened sexual impulses is a phase of the problem which we are not yet prepared to discuss although the importance of this aspect of the problem is recognized.

SUMMARY

By daily injections of 0.62 mg. of testosterone propionate into male rats on and after the ages of 22 to 26 days, the median age of first copulation was set ahead of that of control males by about 20 days. The earliest age at first copulation by an injected male was 29 days; that of an untreated male, 35 days. Probably still earlier copulation may be induced by beginning the injections and the tests somewhat earlier. The pattern of the copulatory act is like that normally appearing in untreated males.

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THE RECOVERY OF TESTES AFTER ANDROGEN INDUCED INHIBITION[1]

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IN 1932 MOORE AND PRICE (1) showed that the administration of an androgenic extract of bull testes to immature rats had an inhibitory effect on the testes. The same effect was obtained by these workers with crystalline androsterone (2), testosterone (3) and testosterone propionate (3). The "grave retardation and injury to the testes of young normal males" thus obtained consisted of gross decrease in size of the testes, reduction in the size of the tubules, sloughing of cells into the lumen and delay in the development of complete spermatogenesis. Similar findings were reported by Sainton, Simonnet and Petresco (4).

While no statement was made as to the after effects of this treatment on the testes, the casual inference was that the damage would be permanent. It seemed pertinent to repeat this work in part and to observe the possible effect of this early treatment on later adult testicular function.

PROCEDURE

Thirteen litters containing 62 male rats were used. Twenty-one males of the first 9 litters were treated daily from the 11th to the 30th day of life. The remaining 19 males were given foster mothers and were placed in separate cages from the treated animals. This was done to avoid possible percutaneous absorption of androgens by the controls from the excreta of the treated animals or from possible oil leakage on the fur of the treated animals. Twelve males of the last 4 litters were treated from the 31st to the 50th day. The 10 controls from these litters were also caged separately.

All treated animals were given 0.1 mg. testosterone propionate[2] daily, administered subcutaneously in 0.05 cc. of peanut oil during the 20-day treatment period. This dosage and period of treatment were used inasmuch as Moore (3) had shown that such a procedure would cause definite testicular injury.

In order to establish that testicular inhibition had taken place, one treated and one control animal from each litter were killed on the day after the last treatment and the testes were weighed. Accordingly 9 treated and 9 control animals were examined after 20 days of treatment at the age of 31 days and 4 treated and 4 control animals after 20 days of treatment at the age of 51 days.

The remaining animals were raised to maturity. In order to test their fertility 2 normal stock females (virgin or post-partum) were isolated with each of the experimental animals and examined weekly for pregnancy. Fifteen of the treated animals and 13 of the controls were living at the age of 9 to 11 months and were killed and

[1] Supported in part by a grant from the Josiah Macy Jr. Foundation.

[2] The testosterone propionate (perandren, Ciba) was furnished through the courtesy of Ciba Pharmaceutical Products Inc.

TABLE 1. IMMATURES

		No. animals	Age at autopsy	Body wt.	Testes wt.	Percentage inhibition
				gm.	gm.	
Group 1	Treated	9	31 days	69 (45–86)	0.187 (0.113–0.246)	67.3
	Control	9	31 days	64 (55–75)	0.572 (0.445–0.728)	
Group 2	Treated	4	51 days	142.5 (103–172)	0.837 (0.270–1.500)	57.4
	Control	4	51 days	138.2 (104–161)	1.966 (1.163–2.105)	

examined 7 to 10 months after the last treatment. At autopsy the testes, seminal vesicles and prostates were weighed; the epididymides were minced in normal saline and examined for motile sperm.

RESULTS

As was expected the daily treatment with small doses of testosterone propionate for a period of 20 days caused inhibition of testicular development in the immatures. The testes of the 31-day-old treated animals averaged 0.187 gm., while those of their litter-mate controls averaged 0.572 gm. (table 1). The testes of the 51-day-old treated animals averaged 0.837 gm., while those of the controls averaged 1.966 gm. (table 1). The testicular weight inhibition was less in the 51-day-old animals (57.4%) than in the 31-day-old animals (67.3%). This difference would be expected on the basis of previous reports (3).

Pregnancies resulted from the matings of only 6 of the 16 treated animals. That this low percentage of fertility was not due to the treatment is established by the fact that, in the same length of time, only 4 of the 13 control males impregnated females. Furthermore, abundant and vigorously motile sperm were recovered from all treated and control animals.

The weights of the various adult organs are shown in table 2. There was no appreciable difference in organ weights between the treated and control groups.

DISCUSSION

While this work was in progress a report by Biddulph (5) appeared. He also

TABLE 2. ADULTS

		No. animals	Body wt.	Testes wt.	Prost. wt.	Sem. ves. wt.
			gm.	gm.	gm.	gm.
Group 1	Treated	9	363 (274–430)	2.775 (2.310–3.340)	0.438 (0.245–0.625)	0.818 (0.448–1.130)
	Control	7	342 (260–404)	2.776 (2.500–3.353)	0.439 (0.360–0.510)	0.960 (0.805–1.025)
Group 2	Treated	6	340 (288–400)	2.829 (2.210–3.358)	0.481 (0.320–0.650)	1.058 (0.800–1.385)
	Control	6	333 (270–380)	2.851 (2.000–3.280)	0.433 (0.345–0.520)	0.826 (0.480–1.060)

produced testicular inhibition in immature rats with small doses of testosterone propionate. In addition, he treated small groups of animals from birth until 51 to 81 days of age and killed them 3 weeks to 3 months later. The testes, seminal vesicles and prostates of the treated animals of these groups were smaller than those of the controls.

In our experiment no such permanent inhibition was found. While these results may seem contradictory, it must be remembered that the procedures used in the two experiments were not identical. In relation to the length of the treatment period, Biddulph allowed a recovery period about one-fifth as long as ours. In actual time his recovery period in all cases was less than one-half as long as ours. Normal male rats, in our colony at least, are sexually mature by 81 days of age. A period of treatment extending from birth through the entire puberal period as in Biddulph's procedure might conceivably cause testicular damage of such severity that recovery would never occur.

With our experimental procedure the inhibitory effect of androgens on the immature testes was demonstrated. This inhibition, however, was only temporary. When sufficient time was allowed for recovery, the testes became normal. This statement applies to both the spermatogenic and endocrine functions of the testes. Complete recovery of the spermatogenetic function was evinced by the presence of normal motile sperm in all animals and by the fact that the fertility of the treated animals was at least as great as that of the controls. That the endocrine function was also completely recovered was evident from the weights of the prostates and seminal vesicles which were equivalent to those of the controls.

SUMMARY

In agreement with previous workers the administration of low doses of testosterone propionate, 0.1 mg. daily for 20 days, caused inhibition of testicular growth in young rats. Seven to 10 months after treatment the testes were normal and no difference was found between treated and control animals with respect to testicular weight, sperm motility, fertility, seminal vesicle and prostate size.

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THE INFLUENCE OF TEMPERATURE ON THE INTERNAL SECRETORY ACTIVITY OF TRANSPLANTED OVARIES IN THE FEMALE RAT

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RECENT REPORTS have indicated that the internal secretory activity of the ovary can be influenced qualitatively and quantitatively by transplantation and temperature regulation. Hill (1) found that transplantation of ovarian tissue into the ears of castrate male mice would prevent the usual castration changes from developing, provided the mice were kept in the proper environmental temperature, 22° C., but not in those animals kept in a warm environment, 33° C. This has been confirmed to some extent by Deanesly (2), and by work which is being done in this laboratory.

Available data are not sufficient to explain this phenomenon. There is some question as to whether the explanation lies in the possibility of the production of an androgen-like substance by the transplanted ovaries or in the androgen simulating action of one of the female hormones. Greene, Burril and Ivy (3) have shown that progesterone in large doses is androgenic.

There is much available data concerning the action of androgens on the genital tract of the female rat and mouse. Salmon (4) brought out the fact that small doses of testosterone propionate when administered over a short period of time produce stimulation of the genital tract. Later, Mazer and Mazer (5) pointed out the atrophy producing effect of testosterone propionate on the genital tract when given over a long period of time.

It seemed of interest therefore to observe the effect of transplanting ovaries into the ears of castrate female rats and subjecting them to the same environmental temperatures as did Hill.

MATERIALS AND METHODS

Animals. White rats of ordinary laboratory stock and of sub-adult age were divided into 3 groups as follows: (*a*) females, castrated and ovaries transplanted subcutaneously into each ear and kept at room temperature, 22° C., (*b*) females, castrated and ovaries transplanted subcutaneously into each ear and kept in a thermostatically controlled incubator at 33° C., (*c*) normal and castrate females placed in the same environmental groups as those of the above 2 groups.

Observations. Vaginal smear studies were carried out on all animals. Miscellaneous observations such as body weight, clitoris size etc., were made. After the desired time had elapsed (55 to 122 days) the animals were killed. The ears of all animals were sectioned and the transplants studied microscopically. The uteri and vaginae were studied both grossly and microscopically.

RESULTS

Group I. This group consisted of 10 animals of which 6 were castrated and their

ovaries transplanted, 3 were normal and one was castrated only. The latter 4 animals served as controls. These animals were placed in a thermostatically controlled incubator at 33° C.

A. Vaginal smear studies. These failed to show anything in the way of a normal estrous cycle. There was, however, evidence of slight estrogenic stimulation as manifested by a sudden decrease in leucocytes and a sharp rise in cornified and nucleated epithelial cells. This effect was transient, however, lasting only a few hours.

B. Postmortem examination. At the time the animals were killed, the uteri of all 6 of the experimental animals were found to be large, well vascularized and normal in appearance. Microscopic examination of the uteri revealed no evidence of atrophy (fig. 1). In all cases there was evidence of ovarian influence. The uteri of 3 of the 6 experimental animals showed evidence of both follicular and corpus luteum hormones, whereas the remaining 3 showed evidence of follicular hormone stimulation only. Microscopically, the ovarian transplants of this group were well developed, well vascularized and showed a preponderance of corpus luteum tissue (fig. 3).

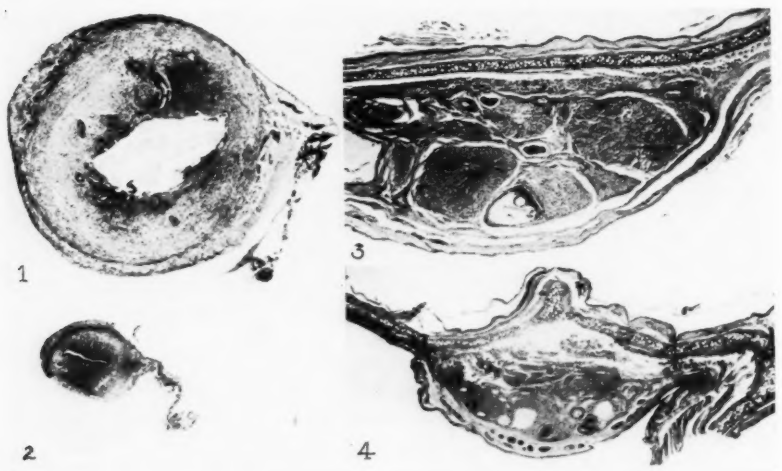

Fig. 1. Cross section of uterine horn of animal *II-A-11*, castrated and ovaries transplanted into the ears and kept in a high environmental temperature, 33° C. Fig. 2. Cross section on uterine horn of animal *II-B-4*, castrated and ovaries transplanted into the ears and kept in a low environmental temperature, 22° C. Fig. 3. Cross section through portion of ear containing ovarian transplant of animal *II-A-11*, a castrated female kept in a high environmental temperature, 33° C. Fig. 4. Cross section through portion of ear containing ovarian transplant of animal *II-B-4*, a castrated female kept in a low environmental temperature 22° C. (all magnifications ×20)

Group II. This group consisted of 13 animals, of which 9 were castrated and their ovaries transplanted, 2 were castrated only, and 2 were normal animals. The latter 4 animals served as controls. This group was kept in a low environmental temperature, 22° C.

A. Vaginal smear studies. These failed to reveal any evidence of estrus except in one case, animal *II-B-7*, which showed evidence of slight estrogenic stimulation.

B. Postmortem examination. At the time of autopsy, the clitora of all animals of this group were enlarged. The uteri of 8 of the experimental animals were avascular, threadlike and atrophic. This atrophy was even more marked microscopically (fig. 2).

There was one exception in this group, animal *II-B-7*, which showed a normal uterus. The explanation for this is not evident. It is believed, however, that some ovarian tissue was left in the abdominal cavity. The ovarian transplants of this group were not as well developed and did not contain as much luteinized tissue as those of the animals which were kept at a high temperature. (fig. 4).

DISCUSSION

The above data seem to indicate that ovaries in the ears of animals which are kept at a high temperature have the power of preventing the usual castration changes in the uterus, whereas those in the ears of animals which are kept at a low temperature do not have this power.

The explanation for this is not evident. There are, however, several factors which should be considered. The first of these is temperature, which, it seems may influence ovarian function in two ways: by directly inhibiting the internal secretory activity, and by interfering with the development of the ovary and thus indirectly influencing the internal secretory activity. Sections of the transplants of the low temperature animals show that the transplants do not 'take' nor develop as well as those of animals which are kept in a high environmental temperature. Therefore, the castration changes which develop may not be due to a complete absence of the ovarian hormone, but rather to a sub-threshold amount of it.

The second factor to be considered involves, as suggested by Hill, the possibility of the production of an androgen by transplanted ovaries which are kept cool by a low environmental temperature. It is well known that androgenic substances such as testosterone propionate when injected into intact female rats over a long period of time will produce atrophy of the genital tract with a suppression of estrus as long as the injections are continued. Since the changes produced in the uterus by this method cannot be distinguished from the usual castration changes, it is impossible to determine whether the atrophy observed here is due to the action of an androgen or to the lack of ovarian hormone. It is important to note that the clitora of all of the low temperature females were enlarged. This has been a constant finding by workers who have injected male hormones over a long period of time.

A third possible explanation lies in the androgen-like action of progesterone. There is much evidence against this. First, large doses of progesterone are necessary to simulate the action of testosterone. Secondly, in this work, there was more luteinization in the ovarian transplants of the animals kept in a high environmental temperature than in those of animals kept in a low environmental temperature. We would expect, therefore, to find more evidence of the corpus luteum hormone in the former group than in the latter one. This is not the case.

It is possible that the atrophy of the uteri of the females which were kept at a low temperature was due to all of the above factors, and that perhaps the effect of a decreased output of estrogenic hormone by the ovaries is potentiated by the action of an androgen or an androgen-simulating substance coming from the same organ.

SUMMARY

Sub-adult female rats were castrated, ovaries transplanted subcutaneously into each ear and divided into two groups. One group was kept in a low environmental temperature, 22° C., the other in a high environmental temperature, 33° C. At the time the animals were killed (55 to 122 days post-operative) the genital tracts of those animals kept in a low environmental temperature were found to be atrophic, whereas those of animals which were kept in a high environmental temperature were essentially normal.

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PHARMACODYNAMIC EFFECTS OF TESTOSTERONE PROPIONATE ON TUBAL CONTRACTIONS (OVIDUCT OF RABBIT) DETERMINED BY CO_2 INSUFFLATION

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IT HAS BEEN ESTABLISHED that in lower animals the spontaneous contractions of the fallopian tubes, like those of the uterus, are governed by the hormonal status (1–5). The tubal contractions of the pig were reported to vary characteristically during the different phases of the estrous cycle. Observations were made upon isolated strips and rings of the oviduct muscle and of the whole extirpated oviducts kept in a state of temporary survival. Rhythmic contractions have also been demonstrated in the extirpated human fallopian tube by Seckinger and Snyder and by Rubin.

By means of uterotubal insufflation a dynamic method has become available for studying the function of the intact fallopian tube both in the extirpated specimen as well as in vivo. Whitelaw (6) confirmed the findings of earlier investigators and demonstrated by tubal insufflation the cyclic variations in tubal motility in the excised sow's oviduct. Cyclic variations found by clinical use of this method have been demonstrated by Rubin (7).

A correlation of the contractions and changes in morphology of the fallopian tubes of the rabbit was made by Wimpfheimer and Feresten (8). Using the technic of uterotubal insufflation they observed the changes in castrated rabbits, castrated rabbits primed with estrogen alone and later in combination with progesterone. Castration was followed by marked reduction in the frequency and amplitude of the tubal contractions. The contractions could be restored to normal levels by the administration of estrogens. The rate of tubal contractions was reduced when estrogen injections were followed by progesterone; the amplitude of the contractions however was unaffected. This method of assaying the effects of estrogen was considered in connection with other hormones as first suggested by Morse and Rubin (9).

In the present report testosterone propionate was used in order to determine its effect on the contraction of the rabbit's oviducts.[2] Experiments with testosterone on segments of surviving rabbit's uterus have been reported by Robson (2) who found that this hormonal substance reduced the motility of the uterine segments. He also found that the response to pituitary hormones was inhibited. This inhibition was present despite the absence of progestational changes in the endometrium. The latter varied greatly.

In our experiments the oviducts of 10 mature rabbits weighing between 3 and 4 kg. were insufflated before and after the administration of testosterone propionate. The following technic was employed. The animals were anesthetized with intravenous injections of nembutal one grain to 2.2 kg. of body weight, and were laparot-

[1] Research Fellow under Dr. Hiram N. Vineberg Grant.

[2] The morphological changes that took place in the oviducts will be presented in a future publication.

omized. A small incision was made in the uterine horn just proximal to the uterotubal junction. Through this a glass cannula was inserted into the uterine cavity and securely tied in place. The oviduct was then insufflated with CO_2 and the tubal contractions were recorded on the kymograph of the Rubin apparatus. The CO_2 was delivered under a constant pressure of 15 lb. and at a constant rate of flow of 1 cc. per second.

Before injecting testosterone, patency of the uterotubal junction was established by insufflation at pressure levels varying between 60 mm. Hg and 130 mm. Hg. The contractions oscillated at pressure levels between 40 and 60 mm. Hg and the amplitude of the individual contractions varied from 5 to 20 mm. Hg.

Following the preliminary insufflation the rabbits were injected on alternate days with 25 mg. of testosterone propionate (neo hombreol). Some of the animals were insufflated after the administration of a total of 100 mg. or 150 mg., and all were insufflated after the administration of a total dosage of 200 mg. of testosterone propionate. There were slight changes after 100 and 150 mg. After a total dose of 200 mg. a notable change took place. This manifested itself by a marked reduction in the pressure required for the CO_2 to pass through the uterotubal junction. The levels of pressure were now found to be between 15 and 30 mm. Hg in all but 2 animals, which required a pressure of 35 mm. Hg. The general level of tubal contractions was now

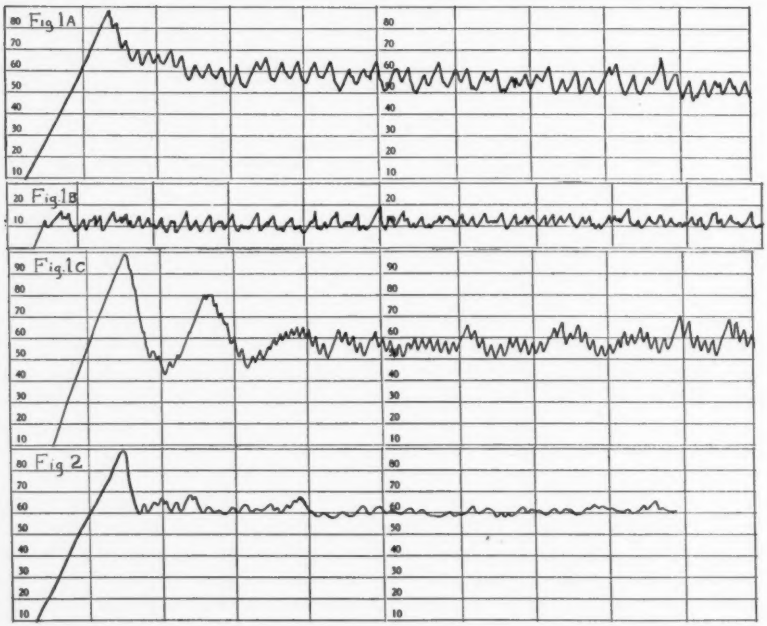

Fig. 1. A, Graph of preliminary tubal insufflation of an anestrous rabbit before injection. Pressure levels between 50 and 60 mm. Hg. B, Graph of tubal insufflation after the injection of 200 mg. of testosterone propionate. Note the low pressure level at 10 mm. Hg. C, Graph of tubal insufflation of the same rabbit (cf., A and B) following the injection of 2000 I.U. of estradiol benzoate. The pressure levels are now between 50 and 70 mm. Hg and the frequency of contractions is increased. Fig. 2. Graph (for comparison) following castration in a rabbit showing low amplitude of individual oviduct contractions at a general level of 60 mm. Hg (after Feresten and Wimpfheimer).

seen to oscillate between 5 and 20 mm. Hg above the zero line (fig. 1). The amplitude of the contractions varied between 2 and 10 mm. Hg. The frequency of the contractions, however, was not significantly altered.

The striking change that apparently took place after the injection of a total of 200 mg. of testosterone propionate was seen first in the low initial pressure required to establish patency of the uterotubal junction; and secondly the low general pressure levels at which tubal contractions were maintained (table 1). It is noteworthy that the loss of tonicity was more marked after use of testosterone than that which was found by Wimpfheimer and Feresten in rabbits after castration (fig. 2).

TABLE 1

Animal	Pressure level of tubal patency		Pressure range at which tubal contractions are maintained above zero level		Amplitude of contractions	
	Preliminary insufflation	Following testosterone propionate, 200 mg.	Preliminary insufflation	Following testosterone propionate, 200 mg.	Preliminary insufflation	Following testosterone propionate, 200 mg.
	mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
1	80	15	50–60	10	5–20	5–10
2	70	20	40–50	5–10	5–15	3– 8
3	90	15	50–60	10–15	5–15	2– 8
4	130	20	50–60	15	5–15	2– 5
5	60	28	50	10	5–15	3– 5
6	70	35	40–50	15	10–15	5–10
7	65	35	40	10	5–20	2– 5
8	75	20	40	20	10–15	5– 8
9	80	25	50	15	5–15	3– 5
10	65	15	50–60	10	5–10	2– 5

In order to see whether the tubal tonicity and contractions could be influenced by estrogen, 5 of the animals which had had injections of testosterone propionate were later injected with estrogens in divided doses. The total dosage varied between 1000 I.U. and 10,000 I.U. over a period of 10 to 12 days. The tubal contractions were now found to resume normal pressure oscillations and in some the initial pressure required to overcome the uterotubal junction was higher than in the untreated animal.

The reduction of the force of tubal contractions of the rabbits' oviducts as determined by uterotubal insufflation in 10 rabbits subjected to injection with testosterone propionate was so uniform as to warrant the conclusion that this hormonal substance has an inhibitory effect upon the tubal muscle diminishing its general tonicity and the amplitude of the contractions.

We are indebted to Hoffman La-Roche, Inc. for their generous donation of the neo hombreol (testosterone propionate) and other material used in this work.

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CLINICAL AND LABORATORY NOTES

PREPARATION OF THE GONADOTROPIC HORMONE FROM THE ANTERIOR PITUITARY OF THE SOW

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Anterior pituitary gonadotropic hormones and anterior pituitary-like hormones have been prepared from several sources. Original alkaline extracts were prepared by Evans[1] and his coworkers from pituitary glands of sheep. Pregnant mare's serum is another source of the hormone. Many different methods of preparing the APL hormones have been reported [2,3,4] by which highly potent preparations were obtained. Recently Friedman[4] prepared a definite gonadotropic substance from the green leaves of immature oat grass.

The present communication deals with the preparation of the gonadotropic hormone from the anterior lobe of the pituitary of the sow. Two methods are reported, first the preparation with dioxane, and second the precipitation with acetone.

Fresh glands were obtained from a local abbatoir and immediately placed in acetone where they remained for 24 hours. The anterior lobes were then easily separated. These were ground to a fine powder and this powder stored in the refrigerator until used. The potency of the original gland and that of the various extracts was tested by determining the smallest amount which when injected into the circulatory system of the sexually mature virgin female rabbit caused rupture of the graafian follicle. A comparison of the amount necessary to cause ovulation with the amount necessary to cause maturation of the ovary of the infantile rat is being studied.

It was found that a fresh anterior lobe of the pituitary weighed an average of 0.1890 gm. and that of the dehydrated gland an average of 0.0378 gm. Each of the fresh anterior lobes contained an average of 15.2 U.

All of the hormone can be extracted from the dehydrated powder by macerating with 10 volumes of distilled water 3 times, allowing 8 to 10 hours for each extraction. The fourth maceration was found to yield practically no hormone. The 3 extractions were combined and the impure hormone precipitated by methods to be described. The preliminary experiments with dioxane showed more promise than with acetone, the latter being quite unsatisfactory. The hormone remains in solution in 60% dioxane, but as this is increased more and more hormone is precipitated out until with 2 to 3 volumes of dioxane all of the hormone is out of solution. Varying the hydrogen ion concentration alters this procedure. The original combined extracts had a pH of 5.6. This solution was adjusted to pH 8 with sodium hydroxide and 2 volumes of dioxane were added. The pH in all cases was determined by Beckman's pH meter using glass electrodes. The mixture adjusted to pH 8 was placed in the refrigerator over night and the small precipitate formed was centrifuged off. It was found to be inactive. The pH of the resulting solution was then lowered by 2 to 3 tenths of a pH with dilute hydrochloric acid and the mixture placed in the refrigerator over night, in each instance. The inactive precipitate was removed by centrifugation and the resulting solution readjusted. An active fraction was obtained between pH 7.1 and pH 7.3. Further lowering of the pH in steps down to 3.5 yielded no active fractions. The fractions coming out at pH 7.1 to 7.3

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[1] Evans, H. M.: University of California Press, Berkeley, 1933 (Memoirs of the University of California, V 11, 1933).

[2] Elden, C. A.: *J. Biol. Chem.* 101: 1. 1933.

[3] Katzman, P. A., and E. A. Doisy: *J. Biol. Chem.* 98: 739. 1932.

[4] Friedman, M. H., and G. S. Friedman: *Am. J. Physiol.* 125: 486. 1939.

were purified by dissolving in a minimum of water, adjusting to pH 8 with sodium hydroxide, adding 2 volumes of dioxane and then gradually lowering the pH, separating the inactive precipitates by centrifugation until the purified material came out at pH 7.3 to 7.1. From 1 gm. of powdered dehydrated gland 0.0137 gm. of crude preparation and 0.00643 gm. of purified preparation were obtained. Of this purified preparation 0.00056 gm. was the smallest quantity which caused ovulation in the rabbit, showing that 22.5% of the dehydrated gland and 4.3% of the fresh gland are gonadotropic hormone.

The purified hormone was extremely soluble in water, insoluble in ether and most organic solvents and soluble in 60% alcohol. It can be adsorbed in part on activated charcoal, a difference from the anterior pituitary-like hormone prepared from pregnancy urine. Long continued heat destroys the hormone and making the preparation in solution alkaline before injection into the rabbit increases its potency 2-fold. From the solubility characteristics this preparation belongs to group 1 of the classes of organic compounds in Kamm's qualitative organic analysis.[5] Preliminary analysis shows the purified hormone to contain carbon, hydrogen, oxygen, sulfur, a slight trace of nitrogen, and a trace of halogens probably as impurities. When ignited on a platinum foil an alkaline residue was left. Analysis of the hormone for sugars according to the method of Hagedorn-Jensen[6] shows it to contain from 7 to 17% of reducing substances.[7] A commercial preparation of gonadotropic hormone prepared from sheep glands containing 7% of reducing substances. Varying amounts of the hormone up to 50 mg. were hydrolyzed with varying amounts of acid or alkali for varying lengths of time from 15 minutes up to 48 hours and in no instance would the resulting solution cause reduction of Benedict's solution. Analysis of the hormone and that of the commercial preparation by Shafer-Hartman method[8] showed less than 5% reducing substance. In the method of preparing the hormone, saccharides are precipitated and are probably carried along as impurities. Both the hormone and the commercial preparation tested gave a positive Biuret and Millon's test, but a negative ninhydrin reaction.

Precipitation of the hormone from the original extract by 10 volumes of acetone is not complete. If a saturated solution of benzoic acid in acetone is added to the acetone, the hormone is apparently completely precipitated but for some reason much of the hormone is lost or destroyed. This phase of the problem has not been investigated. By using acetone alone and varying the hydrogen ion concentration as in the precipitation with dioxane there is no sharp point at which the hormone can be precipitated. Positive fractions can be obtained at pH 7.6 down to pH 5.6, and many of these fractions are obviously contaminated with protein material.

Summary. A method of preparing a purified gonadotropic hormone from the anterior lobe of the pituitary of the sow by precipitation with dioxane at pH 7.1 to 7.3 is described. One unit of the hormone thus prepared weighs 0.00056 gm.

FURTHER STUDIES OF THE HORMONE CONTENT OF HUMAN TISSUES IN PREGNANCY

Frederic Parker, Jr., and Benjamin Tenney, Jr.

In a previous paper[1] we have reported the results of a series of extractions of human tissues, fetal and maternal, for their estrogen content during pregnancy. We found that the maternal liver and the fetal liver contain more estrogenic substance in pregnancy than the placenta. We assumed the above findings to be added evidence against the placenta being the chief source of estrogenic substances in pregnancy.

[5] Kamm, O.: Qualitative Organic Analysis, John Wiley & Son, Inc. 1923.
[6] Hagedorn, H. C., and B. N. Jensen: *Biochem. Ztschr.* 135: 46. 1923.
[7] Fleischer, G., E. Schwenk and K. Meyer: *Nature* 142: 835. 1938.
[8] Schafer, P. A., and A. F. Hartmann: *J. Biol. Chem.* 45: 365. 1921.

[1] Parker, F., Jr., and B. Tenney, Jr.: *Endocrinology* 23: 492. 1938.

In many of the cases after removing the estrogenic substances we extracted the residual fetal tissue for prolan and injected it into immature female rats. In no case did we get a positive reaction. This finding was of interest in contrast to the large amount of estrogenic substance found in fetal organs. Since then a series of 10 fetuses have been extracted for the prolan content of their livers and, in some cases, adrenals and kidneys. As a control we have titrated the amount of prolan in the placenta in each case. Our technic for the extraction of prolan was as follows. Fresh tissue within a few hours of death was ground finely in a meat grinder. Then with 10 times its wet weight of acetone the estrogens were extracted for 24 hours. This was repeated for another 24 hours with fresh acetone. In the third 24 hours the material was extracted with 2 changes of ether. Then the tissue was dried and ground to a fine powder. Adding 2 times the original wet weight of the tissue of distilled water to the powder, it was placed in an automatic shaker in the cold room for 48 hours. Following this the mixture was centrifuged and the supernatant fluid which contained the prolan was removed. Injections were made into immature female rats by our routine A–Z technic of 5 injections in 48 hours. The rats' ovaries were examined by microscopic sections following the completion of the test.

In table 1 are given the results of the first series. There was no positive test result for prolan in any fetal liver, adrenal, kidney or spleen. Even with titrations as low as 100 R.U. per kg. of wet tissue no prolan was found. The placentas ran from 330 to 2500 R.U. per kg. of wet tissue. This immediately made us doubt the usually accepted idea that the fetal blood contained prolan. We obtained fetal cord blood in 3 cases and were unable to find any prolan in the serum at 100 R.U. per liter. Here again although small traces might be found there is no prolan present in relation to the amount in the placenta. This demonstrates that prolan formed by the placenta is secreted by the fetal cells into the maternal blood stream and in no way enters the fetus. Its function must be definitely on the maternal side.

In the routine extraction of human tissues in pregnancy we have never had a positive result at 100 R.U. per kg. from the maternal liver, spleen, adrenal or kidney. The only organ with which we have had positive results is the uterus itself.

TABLE 1. PROLAN CONTENT OF TISSUE

Case	Fetus	Placenta	Maternal Organs
Eclampsia, 7 mo.	Liver neg. at 200 R.U.	1000 R.U.	
Eclampsia, 6 mo.	Liver neg. at 200 R.U.	1500 R.U.	
Stillborn, 6 mo.	Liver neg. at 333 R.U.	Neg. at 333 R.U.	
Separated plac., 8 mo.	Liver neg. at 333 R.U.	4000 R.U.	
Separated plac., F.T.	Liver neg. at 333 R.U.	660 R.U.	
Maternal, accidental, death, 4 mo.	Liver neg. at 160 R.U. kidneys and adrenals neg. at 160 R.U.		Uterus pos. at 330 R.U.
Stillborn hydrocephalus, 7½ mo.	Liver neg. at 330 R.U., kidneys and adrenals neg. at 330 R.U.	1000 R.U.	
8½ mo.	Liver neg. at 330 R.U.		Liver neg. at 330 R.U.,
Intracranial hem., 1 hr., F.T.	Liver neg. at 100 R.U. spleen neg. at 100 R.U.	2500 R.U.	uterus neg. at 330 R.U.
Maternal, accidental death, 7 mo.	Liver neg. at 330 R.U.	2500 R.U.	Liver neg. at 330 R.U., spleen neg. at 330 R.U.

Further studies as to the presence of prolan in fetal and maternal fluids is given in table 2. In 3 cases the relative amount of prolan in the amniotic fluid, blood serum, placenta and urine was checked. In 3 cases, unfortunately not the same, the amount of prolan in the fetal blood was ascertained.

As reported by other investigators the placenta, maternal blood and urine contain approximately the same amount of prolan. Apparently prolan is secreted into the maternal blood stream by the placenta where it remains until excreted by the kidney. There is apparently no renal threshold for its excretion.

The positive finding of 500 R.U. of prolan in the amniotic fluid in the case of severe toxemia suggests a certain backing up of prolan in this condition. This could be explained by the syncytial degeneration characteristic of toxemia as reported in a previous paper.[2] It is of interest that this was found in the amniotic fluid rather than in the fetus. However, as this was found in only one case it will require further investigation.

TABLE 2. PROLAN IN R.U. PER KG. OF WET TISSUE OR PER LITER OF FLUID

Case	Blood Serum	Placenta	Urine	Amniotic Fluid
1	2500	1000		Neg. at 200
2	2500	2500	2500	Neg. at 200
3 Pre-eclamptic toxemia	1000	2500	1000	500 R.U.
Fetal blood	1. Neg. at 100. 2. Neg. at 100. 3. Neg. at 200.			

Discussion. From the above findings it would seem that prolan in pregnancy is secreted by the fetal cells of the placenta into the blood stream. Prolan probably has a local effect on the uterus and decidua as this is the only maternal organ from which it is recovered in any amount. Prolan is not found in the fetus, fetal blood or amniotic fluid in normal pregnancy. Therefore, it has no definite function in regard to the growth or development of the fetus. The maternal blood stream has an amount approximately equal to that of the placenta, and there may be a metabolic function of prolan in the blood. However, this is confined to the blood and does not effect the maternal organs. The kidney excretes prolan in approximately the same amounts as it is found in the placenta and maternal blood. This finding of prolan only in the placenta, maternal blood stream and urine is in definite contrast to the estrogens in pregnancy. The estrogens are found most highly concentrated in the maternal and fetal livers and adrenals. They are also found in the other organs. They are found in maternal and fetal blood and in the amniotic fluid. The estrogens have a potential function in all maternal and fetal organs and fluids. Prolan is confined to placenta and maternal blood.

Summary. Prolan is found in placenta, maternal blood and urine in approximately equal amounts. It is not found in fetal organs, fetal blood or amniotic fluid in normal pregnancy. It is not found in maternal organs during pregnancy except in the uterus.

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THE MECHANISM AND TREATMENT OF PREMENSTRUAL DISTRESS WITH AMMONIUM CHLORIDE

J. P. Greenhill and S. C. Freed*

A GREAT NUMBER or apparently normal women undergo considerable suffering from a variety of causes during the week or so preceding the menstrual flow. With the onset of menstruation this premenstrual distress disappears. The distress occurring at this time may be due to one or several of the following: headache, nausea, bloating of the abdomen, emotional disturbances of many kinds, edema of the vulva and frank edema of other tissues of the body. Although the disagreeable character if premenstrual distress may, in many women, be sufficiently mild to be tolerated, in others it induces considerable misery. Not uncommonly patients suffer severe psychic alterations, with depression, suicidal tendencies or extreme irritability. This condition has been termed premenstrual tension,

[2] TENNEY, B., JR.: *Am. J. Obst. & Gynec.* 31: 6; 1024. 1936.

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supposedly a distinct clinical entity. We have noted, however, all gradations in severity of symptoms during the premenstrual period and it has been difficult to draw a sharp line between premenstrual distress and premenstrual tension. Due to the similarity in symptoms we have concluded that premenstrual tension is an extreme degree of premenstrual distress rather than a separate syndrome.

The etiology of premenstrual tension has been stated to be an increased amount of estrogen in the blood before the onset of menstruation or an insufficient amount of progesterone secretion in this period. Frank[1] has advocated magnesium sulphate by mouth for the treatment of premenstrual tension on the theory that it would hasten excretion of the excess estrogen by the bowel. Israel[2] has reported excellent results in this condition with progesterone therapy.

Whatever may be the etiology of premenstrual tension or distress we have postulated a single mechanism for the development of the variety of changes which occur at this time. The development of premenstrual distress is, according to this hypothesis, the result of sodium ion retention by the different tissues of the body under the influence of the ovarian steroids. This retention of sodium is associated with an increase in extracellular fluid in the tissues which may be microscopic in amount or may develop into gross edema. The neurological symptoms, under this theory, result from edema of the nervous system, probably the brain, the nausea and bloating of the abdomen result from edema of the gut and the other symptoms arise from the specific organs affected.

Actual proof of this working hypothesis is lacking although many physiological reactions can be presented as support for our conclusions. It is well known for instance, that sex hormones are steroids which have a definite effect on water balance. Estrogens, androgens, progesterone and other hormones are capable of causing sodium ion retention. Zuckerman and associates have demonstrated that estrogens induce an increase in water content of the extragenital tissues of the body as well as the sex organs. Premenstrual edema is a clear example of water retention due to ovarian activity. In this connection many workers have noted the resemblance of the sex steroids to those of the adrenal cortex, a major function of which is to preserve electrolyte and water balance.

On the basis of our hypothesis that premenstrual distress results from tissue edema we have attempted to institute therapy whereby the excess sodium and associated fluid are withdrawn. For this purpose we have administered ammonium chloride by mouth, a salt frequently used for removal of edema fluid in cardiac and renal disturbances, although not in the usual diuretic doses.

The manner by which ammonium chloride causes a withdrawal of sodium is not completely established. Apparently, the ammonium ion is changed to urea resulting in an excess of chloride ion. Sodium in the extra-cellular fluid is utilized to combine with this chloride and is then excreted as sodium chloride by the kidneys. Water is lost from the extracellular spaces following the excretion of sodium ion.

Methods and results. The patients were advised to refrain from adding table salt to their food during the last 2 weeks of their menstrual cycle. They were given at this time 0.6 gm. ammonium chloride 3 times daily. Fifteen patients were treated in this manner. They were apparently normal except for the development of marked premenstrual distress. In several cases, a diagnosis of premenstrual tension would be permissible so severe were their symptoms. Two patients had marked edema of the legs and face and two had vulvar edema to the extent that they were unable to wear underclothing in comfort during the premenstruum.

The treatment was uniformly successful. All the patients were definitely relieved and were highly gratified with the therapy. Gross edema did not occur in the patients who had previously demonstrated it. Distension of the abdomen, nausea and migraine were absent or insignificant during the ammonium chloride therapy. The patients claimed that they were less irritable and not subject to the periodic emotional upsets. All patients stated that there was an improved sense of well-being. A more detailed clinical report will be published later.

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[1] FRANK, R. T.: *Arch. Neurol. & Psychiat.* 26: 1053. 1931.
[2] ISRAEL, S. L.: *J. A. M. A.* 110: 1721. 1938.

This therapy had no effect on two women who had migraine which did not occur at the premenstrual period. Patients with dysmenorrhea, and painful breasts were likewise not relieved. The beneficial results were not permanent but were obtained only so long as the ammonium chloride therapy was maintained.

Discussion. It would have been highly desirable to have made water and salt balance studies on these patients. We were unfortunately unable to do. Control therapy with some other salt is also important. Nevertheless our results have been so uniformly successful that we feel justified in reporting them at the present time without a control series.

Our hypothesis, that premenstrual distress is due to organ and tissue edema appears to be a reasonable explanation for the development of the various signs and symptoms. The relief of these disturbances by therapy which was aimed to remove the extra-cellular fluid strengthens our convictions.

Strauss recently emphasized the relationship of water retention to 'functional' toxemia of pregnancy. This type of toxemia can be aggravated by administering sodium chloride and alleviated by withdrawing this salt from the diet. Taylor, Warner and Welsh[3] recently postulated that toxemia of pregnancy might be caused by an increased amount of the steroids from the adrenal cortex on the basis of their studies on electrolyte balance in pregnancy. On the other hand a possible cause for the electrolyte pattern in toxemia would be an abnormal response to a normal amount of the sex steroids. Similarly either of these may be the mechanism for the development of premenstrual distress, although the latter appears most likely at the present time. On such grounds we have been tempted to term premenstrual distress the 'toxemia' of menstruation.

Summary. It is postulated that the symptoms which occur in many women premenstrually, result from the increase in extra-cellular fluid following sodium ion retention in the various tissues of the body. Therapy with ammonium chloride for the purpose of removing the extra-cellular fluid thus formed is effective in relieving the premenstrual distress or tension.

ESTROGENS OF THE FOWL*

H. W. Marlow and Dan Richert†

THE OBJECT of this investigation was to make a quantitative study of the estrogens found in hen's ovaries, immature ova, follicular membranes, and in fresh-laid yolks. Allen and his coworkers[1] extracted the follicles from hen ovaries with alcohol and were able to show that their extract contained an estrogenic hormone from its effect on the uteri of spayed rats. Their study of fresh egg yolk gave a negative response. They used, however, only 18 yolks. Glimm and Wadehn[2] were unable to detect the estrogenic character, using 10 egg yolks and noting the growth of the uteri of rabbits.

Serone and Montezemolo[3] showed that the estrogenic substance was evident in egg yolk only upon progressive incubation of the egg. Fellner[4] reported that the lipoid fraction of egg gave a positive test for estrogens when rabbits were used. Kopec and Greenwood[5] confirmed the work of Fellner by injecting 5 egg yolks into a poulard over a period of 20 days. This resulted in the growth of feminine feathers in 9 days after the cessation of injections. Ogihara[6] stated that the use of eggs in the diet of young rats caused early opening of the vagina

[3] TAYLOR, H. C., R. C. WARNER AND C. H. WELCH: *Am. J. Obst. & Gynec.* 38: 748. 1939.

* Contribution No. 238.

† From the Kansas State College, Department of Chemisty, Manhattan, Kansas.

[1] ALLEN, E., J. W. WHITTSETT, J. W. HARDY AND E. L. KNEIBERT: *Proc. Soc. Exper. Biol. & Med.* 21: 500. 1924.

[2] GLIMM, E., AND F. WADEHN: *Biochem. Ztschr.* 179: 3. 1926.

[3] SERONE, C., AND R. MONTEZEMOLO: *Ross. Clin. Terap. Sci. Affini.* 34: 109. 1936.

[4] FELLNER, O. O.: *Klin. Wchnschr.* 4: 1651. 1925.

[5] KOPEC, S., AND A. W. GREENWOOD: *Arch. F. Estrolngsmechn. d. Organ* 121: 87. 1929.

[6] OGIHARA, M.: *Mitt. med. Akad. Kioto* 21: 315. 1937; *Nutrition Abstracts and Reviews* 1: 927. 1938.

and appearance of the estrous cycle. Ether extracts of egg yolk were more potent than alcohol extracts in castrate females. Riboulleau[7] found no estrogenic compounds in unfertilized eggs of hen, pigeon or duck. He reported finding 1.5 to 2γ of folliculin per gram of yolk in fertilized eggs. Since there is a lack of agreement among the several investigators, it was decided to make thorough and exhaustive extractions and fractionizations of hen ovaries, immature ova, follicular membranes, and of fresh egg yolks, to determine quantitatively the amount of the estrogens in these sources.

Experimental Data

Extraction of hen ovaries. These ovaries contained colorless follicles not exceeding 6 mm. in diameter. The ovaries were ground fine, placed in 95 % alcohol, allowed to stand over night and decanted through cheese cloth. The residue from this treatment was placed in a continuous extractor as described by Marlow and Groetsema;[8] and the extraction continued for several hours using 95% alcohol. The purification of the extract was essentially that described in a paper,[9] and repeated below.

Extraction of content of immature ova. The membrane was removed from frozen yellow follicles measuring 10 mm. or more in diameter. The residue (follicle content) was then ground fine, and extracted in exactly the same manner as the extraction of the whole ovary.

Extraction of follicular membrane. The membrane, after removal from the frozen ovary, was placed immediately in 95% alcohol, extracted and the extract purified as previously noted.

Extraction of fresh egg yolks. The eggs were collected the same day they were laid, and immediatly the yolks and whites were separated from each other. The yolks were beaten and allowed to flow in a small stream into 95% alcohol which coagulated the proteins. The precipitated proteins were ground fine and extracted and purified in the same way as the other fractions (described below).

Process of extraction. The procedure of extraction was essentially the one described by Marlow and Koch[9] for the extraction of residual ovaries from hogs. Modifications were necessary due to the presence of large amounts of fats and cholesterol.

From 1½ to 2 pounds of the material were placed in a muslin sack and subsequently into the extractor. The solvent used in the first extraction was a combination of the alcohol which had previously covered the ground materials and 95% alcohol. The first extraction was terminated after several hours and the extract was replaced with fresh alcohol. Extraction continued until the returning condensed solvent was colorless and the material in the sack (yolk and follicles) was nearly free from pigments. The alcoholic extracts were combined and the extracted yolk and follicles were extracted with cold ether before they were discarded in a nearly white state. This step was eliminated from the process with the ovaries and the follicular membranes. The ether extract was evaporated almost to dryness and the residue was combined with the alcoholic extracts. The combined extraction liquors were allowed to stand over night in a slightly freezing temperature which hardened the fats and oils. The solution was siphoned from the fats and an equal volume of 95% alcohol was added to them. This was warmed on a hot water bath with occasional shaking and the separation of the solution followed as before. The process of ablution was repeated once more.

These fats were also treated to extract any estrogenic material that might be present in combination. They were heated under a reflux condensor for several hours with an acidified alcohol solvent. The alcohol contained 40 cc. of concentrated HCl per liter. The liquor was separated from the fats and oils by the chilling process which has been described. The fats were washed once with 95% alcohol which was combined with the first extract. The solution was made neutral with alcoholic potassium hydroxide and the procedure from here on was the same as that with the alcoholic extracts as given below.

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[7] Riboulleau, J.: *Compt. Rend. Soc. de Biol.* 129: 914. 1938.
[8] Marlow, H. W., and F. Groetsema: *Endocrinology* 19: 415. 1935.
[9] Marlow, H. W., and P. C. Koch: *Endocrinology* 21: 72. 1937.

The alcoholic extract was evaporated under diminished pressure and the residue was taken up in a small volume of hot absolute alcohol. More fatty materials were frozen out, filtered by suction and washed with several portions of cold absolute alcohol. The filtrate was reduced to dryness under diminished pressure and the residue was dissolved in ether. Three volumes of acetone were added and this was chilled to precipitate the phospholipins. The lipins were filtered from the solution by suction, washed with several portions of cold acetone, and the ether-acetone filtrate was evaporated to dryness under diminished pressure. The residue was dissolved in the least amount of hot absolute alcohol and allowed to stand in the ice box at least over night to crystallize cholesterol and separate any fatty material still present These were filtered by suction, the filtrate was concentrated and the procedure of chilling repeated After several such cholesterol crystallizations, the final residue was dissolved in a measured volume of absolute alcohol and stored in the ice box.

TABLE 1. ESTROGENIC ACTIVITY OF THE EXTRACTS

Source	Estrogenic activity
Fresh egg yolk (alcohol extracted)	5 R.U./kg.
Fresh egg yolk (alcohol extracted) followed by HCl-alcohol extraction	No activity
Contents of immature ova greater than 10 mm. in diameter.	44 R.U./kg.
Follicular membrane	166 R.U./kg.
Follicular membrane in terms of weight of the follicles	6 R.U./kg.
Whole immature ova (calc.)	50 R.U./kg.
Hen ovaries with follicles less than 6 mm. in diameter	37 R.U./kg.

The extract for injection was dissolved in olive oil by adding the oil to a portion of the alcohol solution and then evaporating the alcohol under diminished pressure.

Biological assay. The method of assay employed followed essentially the procedure devised by Kahnt and Doisy.[10] The animals used were albino rats of the Wistar strain. They weighed about 200 gm. each.

Vaginal smears were made to determine the regularity of the estrous cycle. Those having regular cycles were bilaterally ovariectomized 2 weeks before the first date of injection. Only those giving a diestrous smear for 5 consecutive days prior to use were kept for assay work.

An approximate estimation of the activity of the prepared extract was obtained before a rat unit was accurately determined. For this a series of single subcutaneous injections of varying amounts were made. To determine the rat unit an amount of the extract estimated to produce estrus in all rats was injected into each of 5 rats. The dose was administered in a series of 3 subcutaneous injections at 4-hour intervals. Injections of decreased dosages followed and the least amount able to produce full estrus in 4 out of 5 rats in 48 to 60 hours from the time of the first injection was considered to be one rat unit.

Estrus was determined by making vaginal smears with moistened swabs of cotton. These were pressed out in a few drops of 0.9% salt solution on a glass slide for observation under the low power of a microscope. The complete disappearance of leukocytes from the field and their replacement by squamous cells indicated estrus. The results of the assay of the various purified extracts are shown in table 1.

Discussion. The results from this research have a bearing on the estrogenic content of hen ovaries, content of immature ova in the final stages of growth of hen follicles, follicular membranes, and of extracts of fresh egg yolk. Estrogenic material was found to be present in egg yolk in small amounts. The small value perhaps indicates the necessity of using larger amounts of the yolk to identify its presence and probably explains the negative results reported by others using small amounts. The follicle contents (immature ova) contained over 8 times as much estrogenic material per unit weight as the fresh egg yolk.

The results of the study of the estrogenic content of the ovaries, and the follicles indicate that the amount present in a laying hen is small. The average weight of the ovaries and

[10] KAHNT, L. C., AND E. A. DOISY: *Endocrinology* 12: 760. 1928.

follicles in a laying hen is less than 100 gm. Then on the basis of the findings that the follicles contain about 50 R.U. per kg. and the ovaries with immature follicles contain about 37 R.U. per kg., the total amount in the ovary of the hen is less than 5 R.U. However, this statement is based on the assumption that no estrogenic material is present in other parts of the body, or is in some combination not released by our procedure. Further investigation is necessary and is being planned to throw more light on the problem.

Summary. The yolk from freshly laid eggs contained 5 R.U. of estrogenic material per kg. of yolk. Contents of immature ova greater than 10 mm. in diameter contained 44 R.U. of estrogenic material per kg. The follicular membranes contained 6 R.U. of estrogenic material per kg. of whole immature ova. Whole ovaries containing follicles less than 6 mm. in diameter contained 37 R.U. of estrogenic material per kg. of ovaries.

THE RELATIVE EFFECTS OF PROGESTERONE AND TESTOSTERONE PROPIONATE UPON OVIPOSITOR LENGTHENING OF THE FEMALE BITTERLING*

Israel S. Kleiner†

RECENTLY de Wit[1] published a note on the relative effects of testosterone propionate and progesterone upon the female bitterling's ovipositor. His conclusion is that progesterone is about 125 times as effective as testosterone propionate. Since several communications from this laboratory[2] have indicated that the ovipositor lengthening is caused chiefly by 'male' hormones (although theelin, theelol, cholesterol and sodium taurocholate have very slight action[3]), it was necessary to examine de Wit's work critically. From the graphs published "it appears that a lengthening corresponding to 1 A.U. is reached within 6 hours by the addition of 0.25 mg. of testosterone propionate or about 0.002 mg. of progesterone to the same quantities of water." The progesterone determination at this dosage was not made but was estimated from the graph. One A.U. is de Wit's unit of measurement, and is 1/6 the length of the first ray of the anal fin. There is no doubt that under these conditions and using these criteria, progesterone is very much more powerful than testosterone. We have repeated de Wit's work and, in a general way, can confirm these results. However, we may say that this comparison has slight justification.

In our experience, testosterone does not show its maximum effects until 24 hours or more after addition to the water of the aquarium, the pure crystalline substances being even slower (up to 72 hours or more) than the male hormones occurring naturally in urine or fractionated from urine. De Wit[4] has found for testosterone propionate a latent period of about 1 hour and an appreciable reaction in 6 hours, but in our many tests we have seldom seen an appreciable reaction before 24 hours. Progesterone, on the other hand, has its maximum reaction probably within 6 to 8 hours, and after 24 hours the ovipositor begins to regress. The two substances, therefore, should not be compared as to their relative potencies at corresponding times.

We have defined[2] a 'bitterling unit' of male hormone as that amount which will cause an increase of at least 7 points in the length of the ovipositor under standard conditions. (On our scale, the anal fin ray is considered 10, rather than 8 as in de Wit's measurements.) In other words we only take into consideration gross changes in the length of the ovipositor. De Wit is concerned with minimal changes. None of de Wit's results are greater than 5.6 points on our scale and most of them are less.

* Aided by a grant from the Lucius N. Littauer Foundation, Inc.

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[1] DE WIT, J. J. D.: *Endocrinology* 24: 580. 1939.

[2] KLEINER, I. S., A. I. WEISMAN, D. I. MISHKIND AND C. W. COATES: *Zoologica.* 21: 241. 1936; KLEINER, I. S., A. I. WEISMAN AND D. I. MISHKIND: *J.A.M.A.* 106: 1643. 1936; *Am. J. Obst. & Gynec.* 33: 458. 1937.

[3] KLEINER, I. S., A. I. WEISMAN AND D. I. MISHKIND: *Proc. Soc. Exper. Biol. & Med.* 34: 367. 1936.

[4] DE WIT, J. J. D.: *Klin. Wchnschr.* 17: 376; 660. 1938.

De Wit finds that from 4 to 30γ of progesterone in 250 cc. of water causes minimal increases within 5 hours. We have used concentrations equivalent to 8, 30, 56, 75, and 94γ in 750 cc. and found slight reactions within 7 to 8 hours and slightly greater ones at about 24 hours,‡ but in no case was there a lengthening which would indicate a unit, that is, there was never a significant lengthening. Testosterone propionate was tried in dosages corresponding to 56, 75, 94, 130, 170, 200, 280, 510 and 1100γ in 750 cc. of water. Occasionally a slight reaction was seen in 6 hours, but the maximal reaction was noted well after 24 hours. A 'Bitterling unit' reaction was observed only when 130, 170 and 200γ were present and then only after 3, 4 and 4 days, respectively. Lower and higher dosages, although showing some effect, did not give what we considered a significant reaction.

We can confirm the fatal effect of 2 mg. of progesterone per 750 cc. of water, on the female bitterling. It is evident that the two bitterling tests are more distinct than de Wit claimed. That is, 'male' hormones have no significant effect during the first 6 to 8 hours and the effect of progesterone is greatest (although not as great as the maximum given by the 'male' hormones) during just that period. Later the reverse is the case, the 'male' hormones' effect continuing to increase while that of progesterone is waning. However, at no time and at no dosage tested did progesterone produce the equivalent of the effect of a 'bitterling unit' of 'male' hormone.§

Summary. Although progesterone causes lengthening of the ovipositor of the female bitterling in greater dilution than does testosterone propionate, this effect is relatively slight as compared with that of the male hormone. Moreover the maximum progesterone action takes place during the first 6 to 8 hours while that of testosterone is observed only in 24 to 72 hours.

THE EMPTYING TIME OF THE HUMAN STOMACH AFTER THE ADMINISTRATION OF PROGESTIN*

Clark K. Sleeth and Edward J. Van Liere†

PROGESTIN, or corpus luteum hormone, has been used in various conditions because of its effect on uterine musculature. Elden and Wilson[1] reported partial or complete relief from dysmenorrhea in a large percentage of selected cases. Since the report[2] that corpus luteum extracts inhibit estrous motility of the uterus in rabbits, and prevent uterine motility after intravenous injection of theelin, many workers have been successful in the treatment of threatened and habitual abortion with this preparation.[3] It has also been noted that the afterpains characteristically following normal parturition in multiparas may be eliminated in about 90% of the cases by a single dose of progestin.[4] Lubin Clark and Reynolds present evidence that the relief of pain is probably associated with the prevention of an ischemic condition of the muscle.[5]

Because of the fact that all investigations to date have been restricted to the action of the preparation on the uterus it was felt that it would be of interest to study its effect on the emptying time of the stomach. It was believed that this might shed some light as to whether

‡ All the experiments reported here were carried out late in the season (April 14 to May 8) and therefore the fish were slower in their responses than they undoubtedly would have been a few weeks earlier.

§ The crystalline progesterone and testosterone propionate used were kindly furnished by Dr. Erwin Schwenk of the Schering Corporation.

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[1] ELDEN, C. A., AND K. M. WILSON: *Am. J. Obst. & Gynec.* 32: 91. 1936.

[2] REYNOLDS, S. R. M., AND W. M. ALLEN: *Am. J. Physiol.* 102: 39. 1932.

[3] FALLS, F. H., J. E. LACKNER AND L. KROHN: *J. A. M. A.* 106: 271. 1936.

[4] LUBIN, S., AND F. J. CLARK: *Am. J. Obst. & Gynec.* 32: 134. 1936.

[5] LUBIN, S., F. J. CLARK AND S. R. M. REYNOLDS: *Am. J. Obst. & Gynec.* 33: 143. 1937.

corpus luteum extract might have some general action, affecting smooth muscle in various sites.

Methods. In 4 healthy male medical students the normal emptying time of the stomach was determined fluoroscopically. The standard meal used for the purpose has been reported previously.[6] Fifteen grams of Farina (Quaker Oats Co.) was added to 350 cc. of boiling water and cooked to a total volume of 200 cc. After the meal had cooled 50 gm. of barium sulphate was added, so that the position of the meal could be determined fluoroscopically. A number of control observations were made for each individual, and the average of the figures was used for the normal in each case.

The effect of progestin was then studied. Twenty minutes before the meal was eaten the individuals were each injected, intramuscularly, with one cc. of progestin in oil. The preparation used was progestin (Upjohn), each cc. containing one Corner rabbit unit in sweet almond oil. Several determinations, exactly at weekly intervals were made upon each individual, and the average was then taken as expressing the emptying time of the stomach of that individual under the influence of progestin.

Results. The results obtained are shown in the accompanying table.

TABLE 1. GASTRIC EMPTYING TIME AFTER ONE CORNER RABBIT UNIT OF PROGESTIN

Subject no.	Normal emptying time, hr.	Emptying time after progestin, hr.	% Change
1	1.92	2.12	+10.4
2	2.00	2.23	+11.5
3	2.17	1.87	−13.8
4	2.08	2.12	+ 2.9
Average	2.04	2.09	+ 2.4

As can be seen from the table there is considerable individual variation in the response to progestin. However, the net result was substantially a failure to influence the gastric emptying time of the subjects as a group. A net change of 2.4% is wholly within the range of the experimental error of the method.

Discussion. From this evidence it would appear that progestin is without any considerable effect on gastric musculature. From numerous reports in the literature[1,4] it must be accepted that progestin is capable of exerting a relaxing effect on the uterus in both the pregnant and the non-pregnant state. Obviously, then, a postulated sensitizing effect of pregnancy is not necessary to explain the quieting of this preparation on uterine musculature. If it is permissible to use the smooth muscle of the stomach as a criterion, it appears that progestin has but little effect on smooth muscle other than that of the uterus. In order, however, to prove definitely the specificity of the substance for uterine muscle the effect of progestin on smooth muscle from various parts of the body should be studied.

Summary. It was found that one Corner unit of progestin (Upjohn), when administered intramuscularly, had no demonstrable effect on the gastric emptying time as determined fluoroscopically in 4 healthy young males. Obviously, then the smooth muscle of the stomach is not affected by this preparation as is that of the uterus.

DYSMENORRHEA RELIEVED BY THYROID

H. Gray*

DEFICIENCY OF THE THYROID as related to menstrual function is obscure. For example, it has been said that "hypothyroidism is commonly related to amenorrhea, but in fact, study of the evidence shows that this is rare and on the contrary the deficiency leads to menorrhagia";[1] and similar non-conformity is found in other recent literature. The topic of this note, the association of hypothyroidism (without abnormal flow) with menstrual

[6] VAN LIERE, E. J., D. H. LOUGH AND C. K. SLEETH: J. A. M. A. 106: 535. 1936.

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[1] YOUNG, J.: A Text-Book of Gynaecology, Black, London, ed. 4, 1936.

pain has been neglected. This phenomenon seems likely however to be commoner than recognized, and since striking relief was obtained by administering thyroid, the following case is reported.

This patient was born December 8, 1921, and has been under regular medical observation all her life. She has always been healthy, with the following trivial interruptions: grippe at 1 year, chickenpox at 3, and tonsillectomy at 4. The menarche occurred early (as was the case with her mother), at 11.96 years, at intervals of 28 days (26 to 30 days), duration 6 days, with first-day pain of very moderate degree. The B.M.R. was first determined because she seemed to her parents slightly overweight and overplacid; no dry skin or falling hair. Both fears have proved transient but the determinations proved interesting in an unexpected connection, namely that of the present note. They were as follows.

At 9.8 years, B.M.R.−13%, pulse 80.
At 13.5 years, Thyroid (Armour) 15 mg. (¼ grain) began.
At 14.1 years, Thyroid (Armour) 60 mg. (1 grain) for about 1 year, then discontinued.
At 14.5 years, B.M.R.−16%, pulse 70.
At 15.7 years, On going to boarding school the first-day cramps became really troublesome, and, on advice of Dr. H. O. Koefod of Santa Barbara, thyroid 1 grain daily was resumed, with such benefit that she has continued it diligently. This relief took place more than a year before the basal rate came up to the theoretical normal.
At 16.0 years, B.M.R.−21%, pulse 76.
At 16.7 years, B.M.R.−14%, pulse 72.
At 17.3 years, B.M.R.+1%, pulse 66.

Conclusion. In dysmenorrhea of unknown cause, which occurs so often, thyroid given to tolerance may afford great relief.

PITUITARY MITOTIC CHANGES AFTER THE ADMINISTRATION OF ESTROGEN AND AFTER OVARIECTOMY

W. C. Kuzell and W. C. Cutting*

EVIDENCE CONCERNING the effect of the ovary on the pituitary is incomplete and somewhat confusing. The problem has been approached both from the standpoint of morphological and of functional effects on the pituitary. Histological investigations[1] have given the impression that ovariectomy reduces pituitary function, while some clinical observations[2] have suggested that the administration of estrin reduces pituitary function. The explanation of this discrepancy may rest in the fact that, although estrin administration causes growth[3] and probably increased hormone content of the pituitary, these hormone fractions may be retained in the pituitary or be other than gonadotropic in action, and thus the apparent function is decreased. An alternate explanation is that in clinical tests, when estrin is used to ameliorate the menopause symptoms, the pituitary, although stimulated to increased growth and function, is outbalanced by the large injections of estrin, with the net result of relatively less pituitary function. Possibly against this view is the finding of decreased amounts of anterior pituitary gonadotropic substance in the urine after estrin therapy.

For additional evidence on this problem we have ovariectomized certain rats, and injected others with estrin for varying periods. Then, on the last day of each procedure, we injected colchicine to mark the mitoses. This enabled us to determine the amount of growth taking place at that time, since mitosis must be considered a sign of growth. Any inference as to function, would, however, be inconclusive.

Methods. Forty-eight virgin female white rats, weighing about 250 gm. each were used. Fifteen rats were ovariectomized on the initial day of the experiment, and 33 normal rats

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[1] SEVERINGHAUS, A. E.: *Physiol. Rev.* 17: 556. 1937.
[2] ALLBRIGHT, F.: *Endocrinology* 20: 24. 1936.
[3] ZONDEK, B.: *Lancet* 1: 776. 1936.

were given daily sub-cutaneous injections of varying doses of estrone, estradiol or diethylstilbestrol.† Nine hours before the termination of the experiment all rats were injected, subcutaneously, with 0.1 mg. colchicine. The pituitary glands were examined histologically, and the numbers of mitoses in 2 cross-sections of the anterior lobe counted. The results obtained are presented in table 1.

TABLE 1. NUMBERS OF MITOTIC FIGURES AFTER OVARIECTOMY AND AFTER ESTROGEN ADMINISTRATION IN WHITE RATS

	Ovariectomized rats			Estrogen treated rats			
Days	No. of animals	No. of mitoses		No. of animals	Total treatment I.U.	No. mitoses	
		Average	Individual			Average	Individual
2				3	1,000 Estradiol	1	(1, 1, 1)
3	2	1.5	(3, 0)	4	1,500 Estradiol	2	(4, 1, 3, 1)
4	1	21.0	(21)	3	2,000 Estradiol	1.3	(0, 3, 1)
5	5	0.6	(0, 0, 0, 0, 3)	3	2,500 Estradiol	1.3	(3, 0, 1)
				1	1,000 Estradiol	2.0	(2)
				1	5,200 Estradiol	12.0	(12)
6	2	1.5	(0, 3)	1	6,400 Estradiol	14.0	(14)
7	1	1.0	(1)	2	3,800 Estradiol	9.5	(17, 2)
8	1	0	(0)	3	13,380 Estradiol	11.0	(13, 15, 5)
9				2	14,940 Estradiol	4.0	(7, 1)
				2	22,500 Stilbestrol	5.0	(4, 6)
11				3	27,500 Stilbestrol	18.0	(41, 7, 6)
14	3	1.3	(2, 2, 0)	1	35,000 Stilbestrol	11.0	(11)
75				1	1,200 Theelin	2.0	(2)
90				1	77,000 Theelin	78.0	(78)
122				1	1,800 Theelin	2.0	(2)
153				1	131,000 Theelin	0	(0)

Results. It is clear that ovariectomy gave no consistent increase in mitosis, while, after a latent period of 4 or 5 days, the injection of estrogens gave a considerable and prolonged increase (table 1). We are indebted to Dr. Laquer of the Department of Obstetrics and Gynecology for the histological slides from animals injected from 75 to 153 days.

The evidences from these experiments indicate decisively that removal of the ovaries is not a stimulus to pituitary growth, and probably therefore not to its function. On the other hand, injections of estrin cause marked growth of the gland, which continued for some time. This probably means that there is also a potential increase in function at least, although, as already stated, this may be concealed by failure of the hormone to be released from the pituitary, or by its being overbalanced by the large amounts of estrin.

The prolonged growth of the pituitary is in contrast to that of the thyroid of animals injected with thyrotropic hormone.[4] In the thyroid, the period of rapid growth is brief, and also the extent of glandular enlargement seems to be somewhat limited. In the pituitaries of estrin injected animals, rapid growth is not only not a brief episode after early injections, but the enlargement of the pituitary progresses over long periods, at times with adenoma formation.[3] It seems to be a definitely less natural and self-limited process than that present in experimental hyperthyroidism.

The inconsistent results after long injection periods presumably mean that, at this time, growth has returned to a normal level, except possibly after the largest doses.

Summary. Ovariectomized white rats showed no consistent evidence of pituitary growth, as indicated by practically unchanged mitotic reactions to colchicine. On the other hand, estrogen injected rats showed a considerable and prolonged pituitary growth, as indicated by definite and sustained mitotic reactions to colchicine.

† The diethylstilbestrol was supplied by the Winthrop Chemical Co.

[4] CUTTING, W. C.: *Endocrinology* 25: 286. 1939.

CURRENT ENDOCRINE LITERATURE

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BOOK REVIEWS

SEX AND INTERNAL SECRETIONS: A SURVEY OF RECENT RESEARCH. Ed. by Edgar Allen, Charles H. Danforth and Edward A. Doisy. The Williams & Wilkins Company, Baltimore, 1939. Pp. xxxvi + 1346.

Because of its scope and masterly command of details this book defies adequate review. Fortunately, however, so well known became the first edition that little more is needed than to call attention to the new. It retains the eclectic thoroughness and sound scholarship that characterized the first edition and incorporates equally good accounts of developments since 1932. To this end, several individual contributors were added to the Staff. While a certain amount of over-lapping is inevitable when a broad field is arbitrarily sub-divided for joint exposition, this defect, if it be defect, was largely obviated by careful initial planning.

As endocrinologists are well aware, fundamental studies in sex research are much in advance of clinical applications. The status of the field is mirrored in the choice of contributors to this volume, nearly all of the 27 being holders of academic rather than medical degrees. In a final chapter, however, the material is brought to a clinical focus by the gynecologist, J. P. Pratt who writes under the caption, "Sex Functions in Man."

The topics treated are those which, by common consent, make up the field of sex research. The 24 chapters are grouped into 5 sections: "biological basis of sex"; "physiology of the sex glands, germ cells and accessory organs"; "biochemistry and assay of gonadal hormones"; "the hypophysis and the gonadotropic hormones of blood and urine in relation to the reproductive system"; "additional factors in sex functions and endocrine applications in man."

In a literal sense, the book is practically indispensable to serious students of the physiology and psychobiology of sex. In addition to the excellence of the subject matter the volume represents a satisfactory piece of book-making, the format being pleasing, the illustrations good and the bibliographies well-selected and easily accessible.

REPORTS ON BIOLOGICAL STANDARDS: VARIABLES AFFECTING THE ESTIMATIONS OF ANDROGENIC AND OESTROGENIC ACTIVITY. C. W. Emmons. Med. Res. Council Special Report Series, No. 234, London, H. M. Stationery Office, 1939.

In this experimental review of selected methods for estrogen and androgen bioassay the author reexamines (*a*) the conditions of assay affecting response, (*b*) animal variation in both mice and capons and (*c*) special factors to be considered in the assay of estrogen-containing extracts.

For estrogen assay oil injections into spayed mice were used exclusively, and curves were set up for the administration of the several natural estrogens in single and multiple dosage; for androgen assay, the effects of injection and inunction were compared in both capons and day-old chicks. Inunction and the use of day-old chicks are both recommended. While variation in responses from time to time shows up in the position (but not slope) of the assay curves, group variation was found negligible in groups of 20 mice. In androgen assay the above statement also holds, but individual variation depends on more recognizable factors, such as age, initial comb size, etc.

From the results of the study several important, but heretofore unemphasized, points are brought out clearly. The substitution of oily for aqueous media cannot supplant multiple administration in the approximation of physiological conditions; this is especially true for the assay of estriol, where 36-hour spread of dosage is essential to bring the level of response up to the order of the more active compounds. Short-cuts in assay have permitted the entrance of inaccuracies in this respect. The presence in biological extracts of the so-called X-substances is to be considered in the light, not of an "augmentatory" action, but of absorption or "protection" of administered hormone; evidence of the latter action is deduced from oral administration of X-substance. The author's conclusion that "we cannot in practice arrive at a trustworthy estimate of the nature or amount of the estrogens present in an impure extract" seem to hinge on the possibility that he has not considered completely the rôle of hormone mixtures in giving the X-effect, i.e., the effect of a slower-acting and (with the assay method used, therefore) much less active compound—estriol, perhaps, in the case of the estrogens. His data lend support to such an explanation.

Although the report by no means purposes to represent a "handbook" of sex-hormone assay, its evaluation of the variables combined in any one assay procedure is of considerable practical value, both in selection of method and in interpretation of results, to any projected application of routine bioassay.

THE ENDOCRINE GLANDS. Max A. Goldzieher. D. Appleton-Century Company, New York, 1939. Pp. 916.

This book represents an evolution from the author's previously published "Practical Endocrinology" which came out in a second edition in 1937. The general plan of the work is similar to that of its predecessor except that the philosophic aspects of the subject are more fully covered. The author has drawn widely on his own experiences, first as a pathologist and then as a clinician to organize the field from both the theoretical and practical points of view. A well-rounded and readable exposition is achieved.

The book is built up on what has become a standard pattern for such undertakings. It begins with a discussion of the general principles of endocrinology, running to ten chapters (64 pages), dealing with such matters as historical aspects, influence of environment, relationship of the vegetative and endocrine systems, heredity and constitution, endocrine interrelations, principles of treatment and so on. Each gland is then taken up seriatim with discussions of morphology and functions, both normal and pathologic. The latter topic is amplified by discussions of the related clinical disorders. Such topics as obesity, asthenia and biology of sex are interpolated in appropriate places in the general schema.

Special attention is given throughout to the diagnosis, management and treatment of the endocrinopathies. It is only in these aspects that there would be any disagreement as to the judiciousness of the work. The author draws freely upon his own experiences and is on the whole optimistic regarding the values of endocrine therapy. The more conservative clinicians may regard some of the therapeutic claims as insufficiently established. Others will attach special importance to the therapeutic discussions as setting forth the experiences of a pioneer in the field. It is the reviewer's impression that a happy medium has been struck between the extravagance of the endocrine impressionist and the nihilism of the over-conservative. Most readers will derive inspiration from the contact with the nascent data without waiting for them to reach their ultimate evaluation and decent burial in conventional textbooks.

The text is well illustrated, largely with original material and is attractively printed. In the various chapters are incorporated well-selected bibliographies which display an eclectic

familiarity with the literature. Those who are still in the primer class will want a somewhat simpler discussion but readers who have attained to any degree of expertness in the endocrine field will find the book one of interest and value.

ANNUAL REVIEW OF PHYSIOLOGY. Published by the American Physiological Society and Annual Reviews. Inc. James Murray Luck, Editor, and Victor E. Hall, Associate Editor. Stanford University, P.O., California, 1939. Pp. 705.

This volume marks the first of a series to be issued under the joint auspices of a volunteer committee and of the American Physiological Society. The purpose of the Annual Review is to present in a broad way the recent advances in physiological knowledge. Each volume is to consist of a group of reviews on selected parts of the field. Of the 24 chapters in Volume 1, the readers of this Journal will be most interested in those on "Growth" by Davenport and his associates; "Energy Metabolism" by Murlin and the "Endocrine Glands" by Evans.

The chapter on growth discusses the hormonal aspects in a page and a half—of necessity, therefore, inadequately. Murlin's chapter includes a well-rounded but not exhaustive discussion of recent researches on endocrine factors in metabolism. Evans, although restricting attention to only the gonads, pituitary and adrenals, required 74 pages for a summary of the 431 recent articles discussed. As he states, the majority of the 2000 or more papers appearing in the year were arbitrarily ignored but excellent judgment was shown in selection and emphasis; thus the total picture presented is representative. The chapter is notably readable. It is concluded that "the need for an 'Annual Review of Endocrinology' is upon us." It seems obvious that if the Annual Review of Physiology is to deal with endocrinology at all less inclusive topics should be selected and more chapters devoted to this field.

ADVANCE ABSTRACTS

ALLEE, W. C., AND N. COLLIAS. The influence of estradiol on the social organization of flocks of hens. *To appear in this Journal.*

In an attempt to find the relation, if any, of estradiol benzoate (Schering's progynon-B) to the peck-order of hens, 13 high ranking individuals among 6 flocks were given daily intramuscular injections. All flock mates received neutral sesame oil. The dosage varied from 0.025 to 1.0 mg. Only 2 reversals resulted although the treatment continued from 39 to 68 days. *BY*, an alpha hen, lost to *BB*, the omega bird in her flock, after receiving 0.1 mg. for 43 days. In another flock *RY*, a medium-ranking bird, lost to *YY*, again the omega bird in her flock, after 36 days treatment with 1.0 mg. daily. All these were in good health. No reversals involving only normal healthy controls occurred during the entire observation period of 10 months. Aggressiveness, as shown by initial staged encounters with strange birds in neutral territory, was little affected by this treatment, if at all. There was no effect even from extended injections of 0.30 mg. or less daily, on egg laying, weight, or size of comb. The comb of *RY*, which received 1.0 mg. daily, became capon-like. She stopped laying but continued to squat before a cock. A poulard began squatting after only 3 days treatment with this dosage and also developed the voice of a normal hen in contrast with untreated poulards. The effect of estradiol treatment as given tends to be opposite to that of testosterone which we reported last year. It is, however, not nearly so striking in its results.

AVERY, T. B., H. M. SCOTT AND R. M. CONRAD. Blood calcium levels of the fowl following injections of theelin. Kansas Agricultural Experiment Station. *To appear in this Journal.*

No increase was observed in the blood calcium value of immature pullets (0.8 kg.) following the subcutaneous injection of theelin (1,750 R.U./kg.) over a 15-day period. Dosages of 1,000 R.U./kg./ /day on the succeeding 4 days or 5,750 R.U./kg. over the 19-day period increased Ca to 14.98 mg./100 cc. of blood as compared with 7.88 mg. for the control group. In a second experiment of 31 days duration 500 and 1,000 R.U./kg./day resulted in a transitory increase on the 7th day whereas blood Ca increased progressively to 40.0 mg. on the 31st day with a dosage of 2,000 R.U./kg./day.

BAKER, A. B., AND C. B. CRAFT. Bilateral localized lesions in the hypothalamus with complete destruction of the neurohypophysis in a pituitary dwarf with severe permanent diabetes insipidus. *To appear in this Journal.*

An 18-year-old pituitary dwarf was studied over a period of 8 years. When first seen she complained of a marked polydypsia and polyuria which continued unaltered until the time of death. She succumbed following an operation for an acute appendix. The autopsy revealed bilateral destructive lesions limited almost exclusively to that region occupied by the supraoptic nuclei. Although most of the tissue destruction was present in the lateral walls of the optic recess, there was some

posterior extension into the anterolateral walls of the infundibular recess involving the medial eminence of the tuber cinerium. There was apparently some retrograde involvement of the paraventricular nuclei since only a few of their cells remained. The neurophypophysis was completely replaced by hyalinized connective tissue—none of the neural elements remaining. The anterior lobe of the hypophysis was partially invaded by connective tissue elements. It was estimated that only about one-sixth of the normal parenchymal cells remained.

BAYER, L. M., AND R. R. NEWELL. The assessment of skeletal development of the hand and knee between the ages of 8 and 14 years. *To appear in this Journal.*

As a contribution to the problem of precision in bone age readings, a study was undertaken of the x-ray evidence of changes in hand and knee at 6-month intervals between the ages of 8 and 14 years. During this period, the diagnosis of bone age according to the methods outlined in Todd's atlas seems most difficult. Ten normally growing boys and girls were each observed by measurement and x-ray every half year for 2 years, starting with a pair at ages 8, 9, 10, 11 and 12 respectively. The resulting 20 sets of hand and knee films could not, on their internal evidence, always be arranged in chronological order. For this age range, therefore, the authors would argue that if developmental changes within the same knee and hand can sometimes not be detected after an interval of 6 months, then the assignment of bone age to a given joint under the usual conditions of a clinical laboratory can hardly hope to be dependable within limits narrower than a year.

BEARD, H. H., AND E. J. JACOB. Creatine-creatinine metabolism and the hormones. III. Effect of parenteral injection of creatine and creatinine with the sex hormones upon creatine-creatinine excretion in normal animals. *To appear in this Journal.*

Different amounts of creatine and creatinine, with and without testosterone propionate and theelol, were injected into normal rats and the effect of this treatment upon creatine-creatinine excretion was observed. The results obtained confirm our previous findings, as follows: Creatine is not changed into creatinine in the rat; the sex hormones injected with creatine likewise do not increase the creatinine excretion. The injection of creatinine stimulates the excretion of creatine and this function of creatinine is increased when it is injected with the sex hormones; no difference in the effect of the sex hormones on creatine-creatinine metabolism in rats has been observed.

BEARD, H. H., AND E. J. JACOB. Creatine-creatinine metabolism and the hormones. IV. Effect of parenteral injection of the sex hormones, with and without saline and creatinine, upon creatine-creatinine excretion in the 90-day castrate rat. *To appear in this Journal.*

Testosterone propionate and theelol, with and without saline and creatinine, were injected into 90-day castrate male and female rats and the effect of this procedure upon creatine-creatinine excretion in the urine was studied. No effect was observed under these conditions upon the excretion of creatine and creatinine which is in contrast to the creatine excreted in the normal animal or recent castrate under similar experimental conditions. The creatinuria observed was due to the effect of saline alone. It was suggested that secondary changes in some of the other endocrine glands may account for these observations. A brief summary of results obtained relating the hormones to creatine and creatinine metabolism is given.

BEILLY, J. S., I. KURLAND AND M. JACOBI. Hormone excretion and bioassay of extirpated tumor in teratoma of the testis. *To appear in this Journal.*

Serial quantitative studies of hormone excretion and determination of the hormone content of extirpated testicular tumor are reported of a patient with malignant teratoma of the testis containing chorio-epitheliomatous elements. In correlating them, it has been suggested that the luteinizing factor found both preoperatively in urine and watery extract of tumor is related to the presence of incompletely developed fetal elements within the tumor. The biological test was of diagnostic value and assay of effectiveness of treatment.

BERGMAN, A. J., J. MEITES AND C. W. TURNER. A comparison of methods of assay of the lactogenic hormone. *To appear in this Journal.*

Three methods of assay of the lactogenic hormone, of increasing sensitivity, depending upon the proliferation of the crop gland of the common pigeon have been compared. Using the McShan-Turner pigeon unit as a standard it was shown that the Reece-Turner method required only 4.5% (1/22) as much hormone and the minimum microunit only 0.56% (1/178) as much hormone to produce a unit response. In comparison to the pigeon methods, 272 McShan-Turner units were needed to induce copius lactation in pseudo-pregnant rabbits. Based upon the best lactogenic preparations, our observations further indicate that the AP factor which stimulates pigeon crop gland proliferation is believed identical with the factor which initiates lactation in mammals.

BERKOW, S. G., AND M. B. JACOBSON. Photoelectric plethysmography of the female breast. *To appear in this Journal.*

Plethysmographs of the female breast, obtained with an improved photoelectric method, are described. Records include studies through the nor-

mal menstrual cycle and during early and late pregnancy, the hands of the same subjects, and male hands. The effects of amyl nitrite and smoking, and of constriction of the breast, are shown. Records made by transilluminating tumors (fibroadenoma and cyst of the breast) are compared with adjacent areas of the same breast. The preliminary investigations suggest several possible applications of this method in studying the circulation of the breast during physiological and pathological changes.

BERNSTEIN, P., AND M. FERESTEN. Estrogenic effects upon tubal contractility and the vaginal secretion in the menopause. *To appear in this Journal.*

Tubal contractions and the vaginal secretion of 24 menopause women were studied before and after estrogenic therapy. A composite graph, suggesting an average value for tubal function in the menopause was obtained by analysis of the records of 80 control uterotubal CO_2 insufflations. In more than one-half of the untreated menopause patients, the tubal function figures were well below those of the composite graph averages. In at least 62.5% of the climacteric patients, tubal function was lower than that found by Rubin in normally menstruating women. In 13 of 19 menopause patients all vaginal smears showed follicular characteristics. The atrophic pattern (low estrogen level) was present in the 6 remaining patients. The untreated menopause patients showed no constant correlation between the type of vaginal smear and the degree of tubal activity. Estrogenic therapy resulted in an increase in tubal function in nearly three-fourths of the menopause patients. In all 6 cases showing the atrophic vaginal pattern, this smear picture was advanced, after estrogenic therapy, to the follicular phase. Only 3 of the 6 patients showed a corresponding advance in tubal function.

BRENEMAN, W. R. Limitation of food consumption as a factor influencing endocrine reactions in the chick. *To appear in this Journal.*

Limitation of food in the chick resulted in a suppression of body growth. When food was available only on alternate days previous to the 30th day, the average body size was only about 50% as great as that of control animals which received food daily. Testes were lighter in the limited-diet birds than in control chicks, both in actual weight and also in proportion to body weight. Although male hormone limited the testes growth in normal diet animals, its effect was not observed in the limited diet chicks. Testes weight of the limited diet and normal diet androgen-treated birds were not significantly different. These facts are interpreted as effects of decreased production of pituitary gonadotropic hormones in limited diet animals. Comb sizes were larger in proportion to body size in the limited than in the normal diet birds. Injection of oreton-B in the limited diet birds produced a greater comb growth than it did in normal diet animals. Injection from the 5th to 9th day in the limited diet series produced a comb growth 50% to 110% greater than in the normal diet chicks. Hormone administration from the 5th to 14th day was proportionately much greater, the limited diet injected birds exceeding the normal diet controls by from 580% to 640%. A study of variability in the series indicates that the limited diet chicks will give a much better test for androgen because of the greater response of the comb.

COCO, R. M. The effects of antuitrin-S, progestin and a vital dye on late pregnancy in the rat. *To appear in this Journal.*

The effects of antuitrin-S, progestin (lipo-lutin), and a vital dye (dianil blue 2R) on late pregnancy in the rat were investigated. A total of 170 animals was employed. Antuitrin-S, injected subcutaneously in single doses of 50 to 75 R.U. on the 16th, 17th, or 18th day of gestation resulted in birth, prematurely or at term, of living, dead, or living and deaf fetuses in 93.9% of the animals. Fetuses born alive were usually not viable. Dosages of 150 to 200 R.U. usually resulted in parturition being postponed and in some instances prolonged from 1 to 5 days. The fetuses died in utero before or at term, and in some cases death of the mothers resulted. Injections made on the 19th, 20th, or 21st day, regardless of the dosage, usually resulted in the birth of viable fetuses at term. Progestin in total doses up to 5 I.U. injected from the 18th to the 22nd day did not appreciably affect the length of gestation or survival of the litter. The vital dye, when injected intraperitoneally in a 1% aqueous solution in daily doses of 4 to 5 cc., from the 14th to the 17th days, inclusive, usually resulted in postponed parturition with resulting dead fetuses. These results are considered to be due to the toxicity of the dye. The physiological phenomena of premature birth, and postponed parturition were correlated with conditions of the ovaries, placentae and uteri as revealed by histological studies. The induced corpora lutea are shown to be nonfunctional. Antuitrin-S is believed to affect the fetuses by interfering with the blood supply through the placentae. Evidence is presented to show that the utero-placental complex plays an important rôle in maintaining and terminating pregnancy.

DILL, L. V., AND C. E. ISENHOUR. An attempt to produce increased susceptibility to renal ischemia in rabbits by means of endocrine preparations. *To appear in this Journal.*

Rabbits receiving varying dosages of several commercial hormone preparations were subjected to renal ischemia by moderate equivalent constriction of the aortas proximal to the points of origin of the renal arteries. Amniotin, eschatin, a saline

extract of the anterior pituitary, follutein, pitocin and pitressin were given 3 times daily by the intramuscular route. Animals receiving pitressin showed albuminuria, depression of the blood pressure and lesions of the kidney, liver, adrenal, myocardium and gastric mucosa when subjected to renal ischemia, while those in which the other preparations had been used showed no significant variation in response from those animals in which only comparable amounts of renal ischemia had been produced. The same clinical course and pathologic lesions were found in animals in which pitressin was given in the absence of renal ischemia. No synergistic effect of these preparations on the course of animals subjected to renal ischemia could be demonstrated.

Donahue, J. K. The occurrence of estrogens in the ovaries of certain marine invertebrates. *To appear in this Journal.*

It was found that alcohol, acetone, ether extracts of the ovaries of four Bermuda forms contained sufficient amounts of estrogenic substance to cause considerable growth of the vagina of mature, ovariectomized rats. The ovarian tissues were taken from the common Bermuda sea urchin (*Lytechinus varieagatus*), the reef urchin (*Echinometria*), the holothurian (*Stichopus mobii*) and the lobster (*Palinurus argus*). In some cases the growth of the vaginal epithelium approximated that achieved with standard estrogens. Dosages of 0.4 cc. of extract daily were particularly effective when subcutaneous injections were alternated with applications of extract directly to the vagina. The possible significance of these estrogens in invertebrate gonads is discussed.

Dorff, G. B. A study of the effects of chorionic gonadotropin on height and osseous development in boys under 8 treated for cryptorchidism and genital underdevelopment. *To appear in this Journal.*

Nine young boys treated chronically and intensively were followed up for several years after the termination of treatment, and studied for the effects of hormonal therapy on growth in height and progress in osseous development. Chorionic gonadotropin in boys under the age of 8 years shows in most of these a definite acceleration in height, and advance in osseous development. There was no evidence of premature union of the epiphyses in these young boys in spite of the production in a few of some precocious secondary sexual characteristics.

Evons, L. T., and E. Hegre. Endocrine relationships in turtles. The effects of seasons and pituitary extracts on the thyroid. *To appear in this Journal.*

Twenty-seven adult turtles (collected within 30 miles of Missoula, Montana) were used. Seven females and 3 males served as controls; 10 females and 3 males received from 4 to 20 daily injections of beef whole pituitary extract. They were killed 24 hours after the last injection. Four females received an average of 36 injections and were killed between 81 and 136 days after the last injection. The injections uniformly decreased the size of thyroid follicles and increased the height of the follicular cells. The after-effects of the injections showed a lack of refractoriness; the degree of hypertrophy was about the same as in turtles killed 24 hours after the last injection. Thyroids collected in midwinter were of the inactive sort typical of hibernating forms, with low cuboidal follicular epithelium and marked colloid storage.

Gardner, W. U., and J. Van Heuverswyn. Inhibition of pelvic changes occurring during pregnancy in mice by testosterone propionate. *To appear in this Journal.*

Testosterone propionate was injected subcutaneously in mice at the rate of 1.25 mg. daily or every other day from the 11th to 13th day or from the detection of the placental sign to the end of pregnancy. The interpubic ligaments of those mice receiving the larger doses or smaller doses for longer periods were largely but not completely inhibited. Some mice died with macerated fetuses in the cervix and upper vagina and 4 mice gave birth to macerated fetuses. Other mice with similar restricted pelvic changes gave birth to living young. Large amounts of androgen are required to inhibit the pelvic changes during pregnancy.

Gershon-Cohen, J., H. Shay, K. E. Paschkis and S. S. Fels. Urolithiasis in the rat after injection of testosterone propionate. *To appear in this Journal.*

Urolithiasis developed in 14 of 46 female rats injected with testosterone propionate in dosages varying from 50 to 200 mg. over periods from 6 to 15 weeks. Control observations were made of litter mates. No calculi developed in a similar number of male rats injected with the same doses during similar periods. The stones were composed of calcium phosphate. No adequate explanation for the findings has been formulated, though several possible mechanical and chemical possibilities were considered.

Glass, S. J., H. J. Duel and C. A. Wright. Sex hormone studies in male homosexuality. *To appear in this Journal.*

To extend previous sex hormone studies in homosexuality, 17 male homosexuals and 31 normal men were subjected to sex hormone assay. The findings are highly suggestive of significant differences in the androgen: estrogen ratio of the homosexual group. Higher estrogenic and lower androgenic values were found in the majority of homosexuals.

GOLDMAN, D. Effects of large doses of protamine zinc insulin in non-diabetic individuals. *To appear in this Journal.*

The effects of large doses of portamine zinc insulin in 24 non-diabetic schizophrenic patients were studied over varying periods from 16 to 98 days. The dosage varied from 50 to 300 U daily. The chief findings of interest were 1. No significant change in sugar tolerance. 2. Low blood sugar without symptoms of hypoglycemia was present a great portion of the time. 3. Marked fluctuation in the blood sugar levels from hyper- to hypoglycemic levels was often found. 4. All of the patients gained significant amounts of weight. 5. Mental improvement occurred in many of the patients but not as satisfactory as that produced by insulin shock therapy. 6. Electrocardiographic studies showed changes similar to those seen under insulin shock treatment.

GRAFFLIN, A. L. Cytological evidence of secretory activity in the mammalian parathyroid gland. *To appear in this Journal.*

The parenchymal cells of the parathyroid gland in the Virginia and Barasingha deer commonly contain numerous small basophilic granules. The pictures observed are very striking when compared with the usual appearance of the 'principal' cells in man and other mammals, and suggest a granular secretory mechanism for the elaboration of the hormone.

HARB, J. F., AND J. R. LISA. The rate of occurrence of hypoglycemia. *To appear in this Journal.*

Because of the lack of statistics relating to the rate of occurrence of hypoglycemia an investigation was made of the routine fasting blood sugars taken at the New York City Hospital, Welfare Island, New York, during a period of 7 years. Records of 21,000 estimations were found. These represent about 1/3 of the admissions to the hospital in that time. The great majority of medical cases were represented as well as those surgical cases that seemed of interest. We found 11% were below 80 mg. and 4% were below 70. Only one of these values was taken during a hypoglycemic shock. There were readings as low as 28 mg. without shocks.

HARTMAN, F. A., AND H. J. SPOOR. Cortin and the Na factor of the adrenal. *To appear in this Journal.*

Methods are described for the separation of adrenal extract into 2 fractions; one which maintains the health of adrenalectomized animals but without effect on Na retention and the other which is responsible for Na retention. It is suggested that the term cortin be limited to the active principle in the first fraction while the term Na factor be applied to the active principle in the second fraction which is responsible for the development of the refractory state. The potency of the Na factor preparation is of the same magnitude as desoxycorticosterone. Methods for assay of the 2 factors are described. The Na factor is present in both medulla and cortex.

HARRISON, H. C., AND C. N. H. LONG. Effects of anterior pituitary extracts in the fasted rat. *To appear in this Journal.*

The injection of saline extracts of beef anterior pituitary into fasting, normal or adrenalectomized rats is followed by a diminished urine nitrogen excretion and ketonuria. At the same time hypoglycemia and a marked reduction in the blood N. P. N. are also found. Fasted, adrenalectomized rats, given large amounts of NaCl and Na_2CO_3 by intraperitoneal injection, develop hypoglycemia, while their urine N excretion is definitely decreased below normal. The association of a decreased N excretion and hypoglycemia suggests that the reduction in blood glucose may be the result of diminished gluconeogenesis from protein due to an inhibition of protein catabolism. The changes in the blood glucose of fasted, adrenalectomized rats may also be explained on the basis of inadequate gluconeogenesis from protein following the loss of the cortical hormone.

HECHTER, O., M. FRAENKEL, M. LEV, AND S. SOSKIN. Influence of the uterus on the corpus Luteum. *To appear in this Journal.*

Pregnant rats were hysterectomized about the 5th day of pregnancy. On the average, these animals went into estrus at about what would have been the 21st day of pregnancy. This reaction appears to be independent of the stage of pregnancy at which hysterectomy was performed. This indicates that hysterectomy of pregnant rats results in the maintenance of luteal function. The functional corpora were inhibited by the implantation of estrous rat uteri. Similar implantations, using diestrous and frozen estrous rat uteri and estrous rabbit uteri, did not inhibit the corpus luteum of hysterectomized pregnant animals. This demonstrates that the uterus is one of the factors regulating the life span of the corpus luteum, through a hormonal or metabolic mechanism.

HOSKINS, R. G., AND S. BEVIN. The effect of estriol glucuronide on the spontaneous activity of senile male rats. *To appear in this Journal.*

Twenty tests were made on 11 senescent or senile male rats of the effect of estriol glucuronide on spontaneous activity. The dosage ranged from 1/2 to 5 cc. of the commercial product, emmenin. It was administered from 3 to 7 times a week, for periods of 3 to 5 weeks. The most common regimen was 2 cc., 5 times weekly. The effect in general was an augmentation of activity. The effect was proved to be repeatable in individual cases. The underlying cause of the augmentation has not been determined.

HOUCHIN, O. B., AND C. W. TURNER. The relation of the anterior pituitary to bile production. *To appear in this Journal.*

The average hourly secretion of bile by 7 female guinea pigs weighing between 280 and 370 gm., taken at a definite time of the day under avertin and ether anesthesia, was found to be 2.94 cc. with a range of 2.5 to 3.2 cc. Following the injection of 25 mg. of an initial extract of the AP, there was a rise in bile secretion extending from 4 to 8 hours. The average hourly production of bile from 13 injected animals was 5.45 cc., an increase of about 85%.

HOWARD, E., AND S. GENGRADOM. The effects of ovariectomy and administration of progesterone on the adrenal x-zone and the uterus. *To appear in this Journal.*

The x-zone of the adrenal cortex in mice is normally well developed in young adult females and in castrated males. It is characterized by a rapid degeneration and disappearance following the injection of androgens, and a similar rapid disappearance of x-zone takes place during the first pregnancy in young adult mice. Estrogens have some tendency to stimulate the differentiation of x-zone. In the present paper an attempt was made to reproduce the effects of pregnancy on the mouse adrenal by the administration of progesterone. Pellets of this substance implanted subcutaneously in normal and ovariectomized females produced no appreciable inhibition of the x-zone. Treatment with pellets combined with injections of progesterone in oil up to 5 mg. daily in a few cases was followed by some decrease in the size of the x-zone, but in other animals the x-zone was similar in size and apearance to that of normal non-bred females, so that one may conclude that the effect of pregnancy on the adrenal x-zone cannot be reproduced by progesterone injections, in doses close to the maximum which can be absorbed. The progesterone treatment used produced marked stimulation of uterine growth in ovariectomized mice, without preliminary treatment with estrogens. The adrenal reaction thus suggests that there is an increase in the physiologically effective level of androgens during the first pregnancy in mice.

HOWELL, K. M., AND S. SOSKIN. Antibody response in rabbits to extracts of human pregnancy urine and to extracts of normal female urine. *To appear in this Journal.*

The complement-fixing antibodies and the precipitins irregularly detectable in the serums of animals which have been injected with extracts of urine from pregnant human females are antibodies to substances present in the urine. They are not specific antihormones in the serologic sense, because similar reactions are produced by urinary extracts that are practically free from hormone.

MCCULLAGH, E. P., AND H. V. LILGA. Bioassays for urinary androgens. *To appear in this Journal.*

Assays in normal men and in cases of pituitary, adrenal and testicular disorders in men are presented. Twenty-four-hour urine specimens were used. Thirty-nine assays were made on 20 normal individuals. They ranged from 18 to 86 I.U. in different cases, and from 23 to 77 U in a single individual. In severe cases of pituitary or parasellar tumors assays ranged from 6 to 59 U. They fell within the range of normal in instances of clinical hypogonadism as well as in cases of impotence. In 5 cases of acromegaly the range was 3 to 93; the high assay occurring in a case of impotence. In 2 cases of Cushing's syndrome assays of approximately 1 and 39 U respectively were found. Five cases, considered Fröhlich's syndrome in individuals 19 years of age or more, showed normal urinary androgens in 2 cases and low androgens in 3 cases. Assays in 27 patients who showed clinical evidence of testicular deficiency are reported. In 4 cases of prepuberal testis injury with evidence of eunuchoidism, assays ranged from extreme low to 33 U. In cases classed as severe functional eunuchoidism, androgen excretion ranged from 0 to 23 U with an average of 3.6. Four cases of mild eunuchoidism showed a range of androgen excretion from 9 to 31 U with an average of 15 U. Five adult castrates excreted from 0 to 18 U daily. Six adults with varying degrees of testicular damage excreted from 3 to 19 U per day. In 3 cases of Addison's disease, urinary androgens were calculated to range from 10 to 45 U in one, and were 20 and 30 U in the other two. Notes regarding the nature of the cases selected are included.

MARBLE, A., A. T. FERNALD AND R. M. SMITH. The effect of human diabetic plasma upon blood sugar curves in rabbits following insulin. *To appear in this Journal.*

The effect of single subcutaneous injections of approximately 10 cc. of blood plasma of diabetic patients upon the blood-sugar-lowering effect of insulin in rabbits was determined. Thirty patients were used as plasma donors. With two exceptions, no significant alteration in the blood sugar curve was observed, even when plasma from middle-aged or elderly obese women with mild diabetes was used. The series included 5 patients with extraordinarily high insulin requirements. When the plasma of 2 of these was used, a definite lessening of the hypogylcemic effect of insulin, subsequently injected, was apparent; this influence persisted for from 3 to 5 weeks. These results could not be duplicated, however, with sera collected from the same two patients at a later date.

NELSON, D., AND R. R. GREENE. Biological indicators of androgenic activity. *To appear in this Journal.*

A statistical analysis has been made of relative merits of the weights of the full and empty sem-

inal vesicles, the ventral prostate, and the vesicular fluid as reliable indicators of androgenic activity in the rat. The study included organ weights of 191 animals, some of which were normal and others treated or untreated castrates. From the analyzed data it was apparent that the weight of the ventral prostate was most reliable when small amounts of androgens were being measured. If the quantity of androgen was equal to or greater than that present in the normal rat, the weight of the empty seminal vesicles was slightly more reliable.

NOBLE, G. K., AND M. WURM. The effect of testosterone propionate on the black-crowned night heron. *To appear in this Journal.*

Both sexes of the night heron, *Nycticorax nycticorax hoactli* (Gmelin), develop a seasonal pigmentation of the lores, lower mandible and buccal cavity; and also a vascularization of the legs which gives them a pink color. Gonadectomy during the breeding season results in a rapid loss of these sexual colors. Injections of testosterone propionate (Ciba) will produce these modifications in gonadectomized adults or in immatures. Estrogens (theelin, estrone acetate, amniotin and follutein) fail to produce any of these changes. Hence, the secondary sexual characters of the night heron are probably under the control of an androgen in both sexes. Both testosterone propionate and the estrogens cause an enlargement of the oviduct of the spayed night heron but only the estrogens produce a hyperplasia of its mucosa. The epididymides and their homologues are hypertrophied by testosterone propionate but not by the estrogens. Testosterone propionate will induce male sexual behavior in adult females or in immatures of both sexes. Even in month-old chicks it will make the voice more guttural and will induce territory defense, nest building, all male courtship ceremonies, copulation and, later, brooding. Differences between the sexual behavior of the adults seem regulated only by differences in the amounts of male hormone normally found in these birds. Estrogens alone fail to stimulate any breeding behavior in either sex.

PINCUS, G., AND M. GRAUBARD. The response of rat uteri to hormone injections. *To appear in this Journal.*

Uteri of immature rats injected with estrogen and excised 6 hours afterwards show a positive correlation between the absolute dry weight and the ratio of uterine wet weight to body weight, which in turn is a function of the injected dosage. Such excised uteri show changes in water content after being kept in isotonic phosphate buffer. Stimulated uteri shrink, while unstimulated ones swell. This transfer of fluids takes place even in non-isotonic solutions and is proportional to the amount of stimulation. Uteri excised 6 hours after injection of estrogen show no or at best a slight increase in rate of O_2 uptake over unstimulated ones. The rate of respiration of all uteri is unaffected by addition of such substrates as glucose or succinate or oxidative carriers such as cytochrome. Respiration is inhibited by iodoacetate and cyanide which indicates that it is due to glycolytic and oxidative processes.

REMINGTON, J. W. Fluid and electrolyte shifts in the normal and adrenalectomized rat after the intraperitoneal injection of isotonic sugar solutions. *To appear in this Journal.*

One hundred six normal and 95 adrenalectomized rats received intraperitoneal glucose and sucrose injections. The response of the adrenalectomized rat differed from that of the normal in the following features. Spontaneous recovery was rare, with the mortality of the order of 80%. None of the injected fluid was excreted by the kidneys. The ascitic fluid volume was always much less, which contributed toward a more rapid absorption of the injected sugar, higher ascitic electrolyte concentrations, and more rapid attainment of equilibrium between ascitic fluid and blood. The dehydration of the blood and extracellular fluid was more rapid in onset, more severe, and showed no relationship to changes in ascitic volume. The serum electrolyte concentrations tended to be lower. Yet far less total electrolyte was shifted into the peritoneal cavity, presumably the key to the smaller ascitic volumes. On the basis of calculation, only 75% of the initial total extracellular electrolyte and fluid have entered into the induced shifts in the adrenalectomized animal. Possible interpretations, in light of somewhat analogous changes in adrenal insufficiency, are discussed.

SCHWARZ, H., A. B. NEWMAN AND H. BAUM. The pituitary function in adiposogenital dystrophy. *To appear in this Journal.*

An investigation of the pituitary function in cases of adiposogenital dystrophy reveals a tendency (not absolute) toward a greater concentration of "ketogenic hormone" in the sera of these cases. Children with Fröhlich's syndrome, however, revealed the same tendency toward induction of ketonemia following fat-test meal, the same sensitivity toward the administration of insulin; epinephrin and toward the Staub-Traugott Effect. No demonstrable amount of gonadotropic hormone was found in the sera of the normal children or those with Fröhlich's syndrome. Genital development was induced in Fröhlich's syndrome cases by administration of gonadotropic hormone. There was no effect upon the obesity.

SCHWEIZER, M., H. A. CHARIPPER AND W. KLEINBERG. Experimental studies of the anterior pituitary. V. Functional activity of anterior pituitary grafts in the adult male guinea pig. *To appear in this Journal.*

Hypophysectomized male guinea pigs with intra-ocular transplants (homoplastic) of anterior

pituitary tissue had well maintained reproductive systems. The occurrence of all stages of spermatogenesis in the testes, the epididymides well filled with sperm and the presence of motile sperm in ejaculates indicated maintenance of the germinal epithelium. Although the interstitial tissue of the testis was less abundant than in the normal guinea pig, the individual cells of this tissue were normal in appearance. There was definite evidence of androgenic activity in the large size and distended state of the seminal vesicles, the size of the horny styles and the positive results of the electrical ejaculation test. The germinal and interstitial tissue of the testis was maintained to the same extent whether the implant tissue was obtained from a male or from a female donor. The bearing of these observations upon current interpretations of certain phases of the gonad-pituitary relation are discussed.

SEALEY, J. L., AND C. W. SONDERN. The comparative action of graded doses of international standard gonadotropic substance (chorionic gonadotrophin) on normal infantile male and female rats. *To appear in this Journal.*

Curves have been presented showing the change in weight of the seminal vesicles, ovaries and uteri from doses of minimum effect to doses of maximum response. Using the ovarian, uterine or seminal vesicle weight changes the potency of solutions containing chorionic gonadotropic factors may be calculated from curves derived from these tables with an accuracy of 15 to 20% through a dosage range of 0.5 to 8.0 I.U. equivalents. Comparison of ovarian and uterine responses to the chorionic standard revealed a striking similarity to the responses of the immature rat ovary and uterus to anterior pituitary and pregnant mare serum observed by others. Histological changes induced in the ovaries, uterus, vagina, seminal vesicles and testes have been described and illustrated with photomicrographs.

SEGALOFF, A., AND W. O. NELSON. Growth of vitamin deficient rats treated with thymocrescin. *To appear in this Journal.*

A purified aqueous extract, thymocrescin, was prepared from calves' thymus glands by the method of Asher. Asher's preparations showed marked growth and gonadotropic effects in rat on deficient diets when injected in doses as low as 1 mg. daily. Our preparations agreed closely with the published chemical analyses performed by the Asher group. No growth or gonadotropic effect could be demonstrated upon 18 rats on a thiamin deficient diet, or upon 8 rats on a horse-meat diet. Attempts to immunize 8 young guinea pigs to thymocrescin were unsuccessful.

SEIDLIN, S. M., The metabolism of the thyrotrophic and gonadotrophic hormones. *To appear in this Journal.*

The aim of this investigation was to discover how far the thyroid and the gonads are responsible for the destruction or inactivation of the specific trophic hormones which act upon them. The experiments are of two main types. In the first group, large doses of a thyrotrophic preparation were injected into normal and into thyroidectomized animals, and the amounts of hormone recoverable from the urine were estimated by biological assay; similarly, a gonadotrophic extract of anterior hypophysis was injected into normal and castrate male and female guinea pigs, whose urines were assayed. In the second group of experiments, potent thyrotrophic extracts were brought into intimate contact with macerated thyroid tissue, which was subsequently removed by filtration, the amount of thyrotrophic activity remaining in the filtrates being determined biologically; similar experiments were performed with gonadotrophic preparations and ovarian tissue; macerated muscle tissue and liver tissue were used as controls of the specificity of the observed inactivations. These experiments are considered to suggest that the thyroid gland plays a considerable part in permanently withdrawing thyrotrophic hormone from the blood, and that the gonads likewise withdraw gonadotrophic hormone from the blood. The experiments with gonadotrophic hormone yielded more decisive results, both *in vivo* and *in vitro*, than those with thyrotrophic hormone.

SMITH, D. C., AND J. M. PERMAN. The effect of thyroxin combined with carotene upon the oxygen consumption of cats. *To appear in this Journal.*

The combined effects of large doses of vitamin A given as carotene (39,000 U.S.P. units daily for 3 days by mouth) and thyroxin (2 mg. daily intra-abdominally) on the basal O_2 consumption, respiratory quotient and weight of male cats was noted and compared with the effect of the same amount of thyroxin when given alone. The carotene definitely reduced the increase in O_2 consumption produced by thyroxin. On the average the basal O_2 consumption of the vitamin A and thyroxin treated animals was only 25.4% of the control value as compared to a rise of 45.74% when thyroxin alone is given. The R.Q. of the vitamin A treated animals was 0.71 as compared to 0.75 in the controls and 0.705 in the thyroxin treated cats. The administration of carotene over this short period of time however did not prevent the loss of weight induced by thyroxin as in both series of experiments the cats lost on the average 5% of their initial weight.

SMITH, R. M., AND B. B. RUBENSTEIN. Adolescence of macaques (*M. rhesus*). *To appear in this Journal.*

The pre-reproductive cycles of 7 female monkeys (*Macacus rhesus*) were observed over a 9-month period. The data were obtained from

vaginal smears, rectal bimanual palpations, observations of the sexual skin phenomena, and the method of controlled-mating with exposure limited to the day of ovulation. It was found that when the vaginal smear method was correlated with the rectal palpation technic the time of ovulation was determined most accurately. In the 6 adolescent monkeys in whom menarche was observed, it occurred at an average weight of 3400 gm., and at a comparative age of 13.1 yr. The pre-conception cycles of these monkeys were extremely irregular, their average duration being 30.6±11.8 days. There were 1 to 4 menstrual cycles before the first ovulation. Conception occurred after the mating at the initial ovulation in each of 4 females but spontaneous abortion terminated the pregnancy in 3 cases. This demonstrated that the period of adolescent sterility may continue after the animal ovulates and can conceive. The duration of the sterile period seemed to be dependent upon the health and growth rate of the animal rather than upon any interval required for sexual maturation. This was illustrated by the fact that the one monkey which carried a normal fetus to term was a robust, healthy animal. The adult monkey was a castrate in whom the normal vaginal smear cycle was reproduced by using estrone and estrone plus progesterone combinations.

SWINYARD, C. A., AND H. D. BRUNER. Compensatory hypertrophy of the dog adrenal gland following unilateral adrenalectomy. *To appear in this Journal.*

Eight male and 8 female dogs were unilaterally adrenalectomized. The gland removed surgically was used as a normal control for comparison with the remaining gland which was removed at autopsy after post-operative periods which varied from 3 to 90 days. The average total volume of the glands removed at autopsy was 13.4% larger than the normal glands removed surgically. The compensatory hypertrophy was confined to the z. glomerulosa and z. fasciculata and averaged 15.6%. The volumetric increase of the outer cortical zones was at the expense of the z. reticularis which decreased in volume by 20.7%. The medulla appeared to be slightly more compact in the hypertrophied glands and in these glands the average volume of the medulla was 3.7% less than in the normal glands. The hypertrophy apparently occurs rather rapidly about the time deficiency symptoms appear in totally adrenalectomized animals and does not change significantly during long post-operative periods.

SZABÓ, I. Hypertrophy in the pseudopregnant uterus of the mouse, following mechanical stimulation or treatment with vitamin E. *To appear in this Journal.*

Out of 27 pseudopregnant mice none showed urerine hypertrophy. To 9 pseudopregnant mice vitamin E was administered, and of these 5 developed uterine hypertrophy. A group consisting of 11 mice were stimulated daily on the cervix uteri with a glass rod for 45 to 58 days; this resulted in uterine hypertrophy in 7 cases. The histology of the hypertrophied uterus showed increased musculature and connective tissue, blood vessels and glands very much regressed, epithelial cells low and leucocytes absent. Administration of vitamin E to normal mice did not result in uterus hypertrophy.

TRUSZKOWSKI, R., AND J. DUSZYNSKA. Protection of mice against potassium poisoning by corticoadrenal hormones. *To appear in this Journal.*

The toxicity of potassium salts to immature mice varies inversely with the amount of cortical hormone in circulation, being greatest for adrenalectomized mice, and least for mice given deoxycorticosterone acetate before injection of K salt. At a given dosage level of KCl percentage mortality is related linearly to the logarithm of the dose of doca previously given. Percentage mortality-log dose curves may serve for the assay of doca preparations, the method being simpler, cheaper, and quicker than any other so far described. Following adrenalectomy the growth curves of mice given appropriate doses of doca approximate to those of normal animals. As assayed by the Bomskov test 1 mg. of doca contained 20 mouse units. The results are in full accord with the view that an important function of corticoadrenal hormones is the regulation of K metabolism.

TURNER, C. W., AND P. T. CUPPS. The effect of certain experimental conditions upon the thyrotropic hormone content of the albino rat. *To appear in this Journal.*

A study of the thyrotropic hormone content of the pituitaries of albino rats of both sexes weighing between 150 and 200 gm. following various periods of gonadectomy is reported. Following gonadectomy, the thyrotropin content of the AP of both males and females was reduced slightly after 20 days and rather markedly after 66 days. Replacement therapy with estrogen at the rate of 40 R.U. and androgen at the rate of 200γ daily appeared to maintain the normal level of thyrotropin in the castrate female but not in the male. Thyroidectomy of male rats for periods of 40 days and 6 months resulted in a reduction of about 50% in the thyrotropin content of the AP. In contrast, females similarly treated maintained their normal content or showed a slightly increased level of hormone.

WILSON, R. H., AND F. DE EDS. The synergistic action of thyroid on fluorine toxicity. *To appear in this Journal.*

The enamel of the normal rat incisor is white at the time of eruption, but quicly acquires a brown-

ish color which rapidly darkens until the deep brown color of the normal adult rat incisor is reached. The presence of adequate amounts of fluorine in the diet produces a bleaching of this color. Young albino rats of approximately the same age and weight were placed on diets containing enough fluorine to cause a very slight degree of bleaching of the incisors. When powdered thyroid was added (0.2% of the diet), a rapid bleaching occurred and the teeth became almost white. The feeding of thyroid caused an increase in food consumption, and consequently an increased fluorine intake. However, this increase in fluorine intake was too small to account for the greater bleaching action. This was proven by observations that subcutaneous injections of protamine Zn insulin, which also increased food intake to an equal or greater extent, did not produce an increased bleaching of rat incisors.

VELASQUEZ, J., AND P. ENGEL. Urinary excretion of gonadotropics in cattle suffering from cancer. *To appear in this Journal.*

The urine of 5 non-pregnant cows, suffering from cancer of the region of the eye, was injected to infantile rabbits. In all cases there was observed follicle ripening and luteinization and in one rabbit hemorrhage in the follicles.

WIGODSKY, H. S., AND R. R. GREENE. The effect of testosterone, estrone and estradiol applied locally to the penis of the rat. *To appear in this Journal.*

The application of 7.5γ testosterone to the penis of castrated immature rats causes a definite growth of this organ in 10 days. This growth is as great as that obtained when the animals are treated with the same daily dose for 22 days. The growth of the penis in rats treated with testosterone in lanolin was less than that obtained with testosterone in alcohol. The daily application of 1γ of estradiol for 22 days or 5γ for 10 days, or the application of 5γ of estrone for 10 days failed to cause any significant increase in the length or weight of the penis of castrate rats.

ABSTRACTS OF ENDOCRINE LITERATURE

ADRENALS

BAKER, D. D. Differential stain for the nuclei of the suprarenal cortical cells. *Anat. Rec.* 74: 401. 1939.

Nuclei of the suprarenal cortical cells in mouse, rat, cat, bat, dog, guinea pig, rabbit, cattle, and man, were differentially stained by applying fuchsin, orange G, and aniline blue separately to chromated frozen sections in gelatin. The nuclei of the cortical cells stained in 4 colors: dark blue, light blue, red and yellow. The blue nuclei were most numerous and yellow the least. The various nuclei were seen in all zones. In the human tissue some differentiation of cytoplasm also appeared, as yellow, greenish-gray, blue-greenish gray, and blue. In a certain type of cell, the cytoplasm stained yellow with orange G. These small cells were compressed, with concave borders. The nuclei were slightly smaller than those of other cells and some showed shrinkage of the nuclear membrane and pyknosis. Prolonged treatment (12 to 14 days) with potassium bichromate, induced some differentiation of the nuclei and cytoplasm of the cells of the medulla of the rat suprarenal.—*Abstract, Wistar Institute Bibliographic Service.*

COREY, E. L. An apparent sex-specificity in the action of progesterone in adrenalectomized cats. *Proc. Soc. Exper. Biol. & Med.* 41: 397. 1939.

Progesterone (proluton) administered to adrenalectomized cats at the appearance of definite symptoms of adrenal-cortical insufficiency was immediately followed by rapid recovery and subsequent return to normal (blood chemistry), notably with respect to normal carbohydrate level, following the post-adrenalectomy depletion. Animals maintained in good health by progesterone treatment subsequently succumbed after withdrawal of treatment. Nonpregnant adrenalectomized cats succumbed despite progesterone dosages. The life-span of pregnant cats was prolonged by the treatment.—E. T. Gomez.

DRYERRE, H. W. Addison's disease. The diagnostic significance of the sodium and chlorine content of blood and urine. *Edinburgh M. J.* 46: 267. 1939.

The test for adrenal insufficiency described by Cutler, Power, and Wilder consists in feeding suspected patients on a diet low in sodium chloride and potassium for 2½ days. In normal individuals the chlorine and sodium excretion decreases while in adrenal insufficiency it remains high. This test was tried on 5 normal individuals, 5 suffering from asthenia not of adrenal origin, and 4 cases of proved Addison's disease. On the third day these latter showed a higher sodium output than either of the other groups. Neither excretion of chlorine nor the serum sodium and chlorine were materially different in the 3 groups. One patient with Addison's disease reacted very unfavorably at the time the test was in progress, so there is a possible danger in its use.—J. C. D.

FLEXNER, L. B., AND A. GROLLMAN, The reduction of osmic acid as an indicator of adrenal cortical activity in the rat. *Anat. Rec.* 75: 207. 1939.

The cytological changes occurring in the adrenal cortex of the rat, as evidence by the reduction of osmic acid, were correlated with changes in the

activity of this gland. Reduction in adrenal activity was induced by the administration of various amounts of the cortical hormone. An increase in cortical activity was produced by unilateral adrenalectomy, heat, cold, and injection of tetrahydro-β-naphthylamine, etc. A definite correlation was found to exist between the degree of change in cortical activity and the nature of the cytological changes as indicated by the reduction of osmic acid. The nature of these changes in the various zones of the cortex are described. In general, depression of cortical activity is correlated with a decrease, while stimulation of activity is accompanied by an increase in the cytological constituents which reduce osmic acid.—*Abstract, Wistar Institute Bibliographic Service.*

GERSH, I., AND A. GROLLMAN. The nature of the X-zone of the adrenal gland of the mouse. *Anat. Rec.* 75: 131. 1939.

When the adrenal gland of the immature or castrate mouse was stimulated by the administration of thyroid powder, which created a need for cortical hormone in the organism, there was a pronounced hypertrophy of the X-zone. The cells of this zone increased in size and their cytoplasm accumulated large numbers of small droplets of lipid that reduce osmic acid. Degeneration of the X-zone almost to complete extinction followed the administration of small doses of cortical hormone to immature or castrate mice. In very young animals, administration of large doses of the hormone may inhibit entirely the appearance of the zone. These results were interpreted to indicate 1) that the X-zone performs the same functions that are carried out by the other two zones of the cortex, 2) that the X-zone acts as a reserve tissue which responds to increased demands on the adrenal glands for their hormone, and 3) that the X-zone is that portion of the cortex which is most readily depressed when a great need for cortical hormone does not exist or is satisfied in some other way.—*Abstract, Wistar Institute Bibliographic Service.*

GOLDZIEHER, M. A. Adrenal cortical disturbance. *Internat. Clin.* 4: 20. 1939.

This is a good review of the subject, which does not lend itself to successful abstracting. There are illustrative case histories of value to the diagnostician, and an ample discussion following each case. The subjects discussed are Addison's disease, hypo-adrenia, hyperinterrenalism, pseudohermaphroditism, precocious puberty, virilism, diabetes of the bearded woman, and Cushing's syndrome. Treatment of these respective conditions is discussed.—I. B.

GROLLMAN, A. The comparative activity of desoxycorticosterone and other crystalline derivatives and of purified extracts of the adrenal cortex. *J. Pharmacol. and Exper. Therap.* 67: 257. 1939.

Corticosterone, desoxycorticosterone acetate, extract concentrate of adrenal glands and Grollman's crystalline adrenal preparation were assayed on groups of adrenalectomized rats. Since desoxycorticosterone (a synthetic product) 1 mgm. daily maintained normal growth in the month-old adrenalectomized rat, it was suggested as a standard for assay purposes. Corticosterone and a number of other crystalline adrenal cortex preparations were found inactive or had little activity when compared with desoxycorticosterone. A crystalline steroid, which is highly unstable, having an activity of approximately 100 rat units per mgm. was prepared from the adrenals.—E. L.

KEENEY, E. L. Epinephrine in oil. Its effectiveness in the symptomatic treatment of bronchial asthma. *Am. J. M. Sc.* 198: 815. 1939.

Twenty patients with chronic bronchial asthma who had been taking frequent injections or inhalations of epinephrine hydrochloride every day and every night for from 2 months to 4 years, received relief from asthmatic symptoms for from 3 to 24 hours with from 0.5 cc. to 1.5 cc. doses of epinephrine in oil. One patient received no prolonged effect from adequate doses. Forty-nine patients were treated during one or more acute paroxysms of asthma and each received from 0.5 cc. to 2 cc. of epinephrine in oil. Forty-eight patients remained free from asthma for 4 to 24 hours. One patient received more satisfactory relief from epinephrine hydrochloride than from epinephrine in oil. The injections of epinephrine in oil were made subcutaneously in the upper arm or intramuscularly in the deltoid or gluteal muscles. In all, 70 patients received 967 injections of epinephrine in oil. One severe reaction to the oil and one severe reaction referable to the epinephrine occurred.—*Author's Summary.*

SCHACHTER, R. J., AND M. O. BEBEA, JR. Assay of adrenal cortical extracts. *Proc. Soc. Exper. Biol. & Med.* 40: 541. 1939.

A technique for adrenalectomy of the guinea pig was described. A 2-stage operation at 2 week-intervals was performed in animals weighing 400 to 600 gm. Adrenal cortical extract was injected beginning immediately after the 1st removal and was continued daily for 10 to 12 days, when adrenalectomy was completed. Withdrawal of cortical extract after the 2nd stage adrenalectomy resulted in death within 3 to 4 days. Death could be successfully averted by adequate replacement with adrenal cortical extract. The amount of extract which when given in daily doses of 1 cc. would maintain a 1 kg. guinea pig in normal health for a period of 10 days was proposed as a unit of activity.—E. T. Gomez.

SWINGLE, W. W., W. M. PARKINS, A. R. TAYLOR, H. W. HAYS, AND J. A. MORRELL. Effect of extract of anterior pituitary on the life-span of

castrate adrenalectomized cats. *Proc. Soc. Exper. Biol. & Med.* 38: 876. 1938.

The administration of anterior pituitary extract significantly prolonged the life-span of bilaterally adrenalectomized cats, although all survivals showed symptoms of chronic adrenal insufficiency. The role of the anterior pituitary in this instance is not clear.—E. T. Gomez.

ENDOCRINE GENERAL

CANNON, W. B. A law of denervation. *Am. J. M. Sc.* 198: 737. 1939.

The evidence is summarized to indicate that the various structures which respond to adrenalin or sympathin and to acetylcholine undergo an augmentation of sensitivity when denervated. The phenomenon is generalized as a law applicable to all reactive tissues and chemical stimulants.—R. G. H.

GONADS

BLUNN, C. T. The age of rats at sexual maturity as determined by their genetic constitution. *Anat. Rec.* 74: 199. 1939.

Significant differences in the mean age at sexual maturity and mean rate of growth from weaning to sexual maturity were found between ♀ ♀ of 2 strains of rats whose parent stocks came from widely separated localities. The reciprocal F_1 populations were early maturing and rapid growing like the early maturing parent. The 4 backcross populations showed some segregation into early and late maturing and into slowly and rapidly growing groups. The data indicated that even though an association was found between rapid rate of growth and early maturity, they were not inseparable phenomena. For the rats studied, genetic composition was of greater importance in determining the age at which the ♀ ♀ became sexually mature than was the size of the litter in which they were raised to weaning time.—*Abstract, Wistar Institute Bibliographic Service.*

BRADBURY, J. T., AND F. GAENSBAUER. Masculinization of female rat by gonadotropic extract. *Proc. Soc. Exper. Biol. & Med.* 41: 128. 1939.

Two R.U. of antuitrin-S or its equivalent of A.P.L. administered daily from 6th day produced definite masculinization in 6 of 8 ♀ rats, as indicated by hypertrophy of the clitorides and development of preputial glands. Similarly, rats receiving 5 R.U. of pregnant mare serum (gonadogen), 10 I.U. of estrone or 3 mg. of testosterone on alternate days showed vaginal patency on day 14 to 16. Five R.U. of pituitary extract did not exert similar effects.—E. T. Gomez.

BURACK, ETHEL, J. M. WOLFE, AND A. W. WRIGHT. Prolonged vaginal bleeding and fetal resorption in the Albany strain of rats. *Anat. Rec.* 75: 1. 1939.

The Albany strain of rats, in addition to an unusually high incidence of spontaneous mammary fibroadenomata in the ♀ ♀, is characterized by low fertility. One factor which contributes to this is the occurrence of spontaneous fetal resorption. In this study 400 pregnancies in the Albany strain and 140 control pregnancies in rats of the Vanderbilt strain, in which tumor incidence is low and fertility is high, were analyzed. The results were compared statistically. Significant differences were obtained indicating that the rats of the Albany strain 1) manifest vaginal bleeding earlier in pregnancy, 2) bleed more profusely and over a longer period and 3) give a lower average of young per little than the control animals, due to the fact that in an appreciable number of rats either some or all of the fetuses are resorbed. Study of the gross appearance of the uteri showed that spontaneous fetal resorption was associated with marked variation in the number of resorbing sites, time of onset of the process, and rate of resorption. The resorbing foci were characterized by hemorrhage into the fetal sites. Histologically, hemorrhage and tissue destruction were noted in the lateral margins of the placenta. Degeneration of fetal tissue was secondary to the placental lesions. Gestation followed by delivery was not in general prolonged. Pregnancies resulting in complete resorptions extended in many instances to 28 days.—*Abstract, Wistar Institute Bibliographic Service.*

BUXTON, C. L., AND E. T. ENGLE. Effects of the therapeutic use of diethylstilbestrol. *J.A.M.A.* 113: 2318. 1939.

In 16 of 17 cases followed over 2 to 6 months following treatment with diethylstilbestrol, no evidence of toxicity attributable to the estrogen could be found. Laboratory investigation included complete blood counts, urinalyses, serum, protein partition, icteric index and van den Bergh reactions. Toxicity possibly ascribable to the estrogen was observed in 1 patient with positive urnary manifestations. No evidence was obtained of allergic or urticarial phenomena. Dosages varied from 1 to 30 mg. daily, orally, for periods of 1 to 8 weeks.—H. O. H.

BUXTON, C. L., AND U. WESTPHAL, Recovery of pregnandiole in urine of men treated with progesterone. *Proc. Soc. Exper. Biol. & Med.* 41: 284. 1939.

Pregnandiole as sodium pregnandiole glucuronidate in amounts averaging from 0.1 to 8 mg. daily was demonstrated in urine of 3 Addisonian patients (2 ♂ ♂ and 1 ♀) and 1 normal ♂ control who received 30 mg. crystalline progesterone daily. In a single instance 20 mg., or 67% of the injected progesterone, was excreted over a 24 hour period.—E. T. Gomez.

CHASE, ELIZABETH B. The reproductive system of the male opposum, Didelphis virginiana Kerr and its experimental modification. *J. Morphol.* 65: 215. 1939.

In the ♂ opossum seminal vesicles, ampullary glands, and a prostate in a separate capsule are lacking. However, accessory glands are present in the form of an enlarged urethra and as well developed Cowper's glands. The glands in the wall of the cranial part of the urethra consist of tubules radially arranged around the lumen of the urethra and emptying into it by a large number of openings. Histological examination showed three different types of glands to be present in this region, each with a distinctive epithelium and limited to a definite region of the urethra. From a study of the gross anatomical relations, histology, and development, the name prostate was considered justified for these glands and the three types designated as prostate I, II and III. The name urethral glands was applied to small glands in the caudal portion of the membranous urethra. Castration effects were noted as a decrease in weight of prostate and Cowper's glands. Histological changes were noted in both types of glands. Injection of gonadotropic hormones into semi-castrate animals caused an increased weight of urethra (including prostate glands) and Cowper's glands, with large doses, but no difference in the testis. No histological change was noted.—*Abstract, Wistar Institute Bibliographic Service.*

Corner, G. W. The ovarian hormones and experimental menstruation. *Am. J. Obst. & Gynec.* 38: 862. 1939.

The author reviews his and other authors' published results which can all be correlated as indicating that menstruation follows the reduction of ovarian hormone effects on endometrium, either of estrin alone, or of estrin followed by progesterone and subsequent reduction of the latter. The flows which follow ovulation and anovulatory changes can be differentiated only by study of the ovaries or endometrium.—E. L. S.

Dempsey, E. W. Maturation and cleavage figures in ovarian ova. *Anat. Rec.* 75: 223. 1939.

Normal and abnormal maturation figures are observed in large numbers in the ova of atretic follicles from guinea pigs killed shortly after ovulation or after injections of luteinizing hormone. Similar maturation figures may be seen, but less frequently, in atretic ova from guinea pigs killed at other times in the reproductive cycle, after daily injections of progesterone and after hypophysectomy. Since progesterone inhibits the release of luteinizing hormone and hypophysectomy abolishes all pituitary gonadotropic hormones, it is concluded that the pituitary hormones are not essential for the initiation of maturation changes in ova.

These ovarian ova may divide and differentiate into quite complex structures. Occasional structures containing giant cells, morphologically similar to trophoblastic giant cells, occur. These structures ultimately undergo degeneration and are reabsorbed.—*Abstract, Wistar Institute Bibliographic Service.*

Dorr, E. M., and R. R. Greene. Treatment of the menopause with estradiol dipropionate. *Am. J. Obst. & Gynec.* 38: 458. 1939.

In 55 menopausal women the authors found it possible to secure optimal control of symptoms by intramuscular injections of this ester at 1 to 4 week intervals, in varying doses. In 11 of these they substituted the same weight of estrone, and in 10 cases it was obvious that the estradiol ester was more effective.—E. L. S.

Emerson, Gladys A., and H. M. Evans. Restoration of fertility in successively older E-low female rats. *J. Nutrition* 18: 501. 1939.

The reproductive performance of successively older vitamin E-low female rats permits us to recognize three periods.

1. From the age of 3 to 6 months, at which time the response to a single dose of 0.5 gm. of wheat germ oil invariably leads to the birth of normal sized litters of living young. Only this period can be recommended for assay studies.

2. From the 7th to the 12th month, at which time the response is variable; in the earlier part of this period only half the animals give birth to living young with this dosage (0.5 gm.) and these are under-sized litters; moderate elevation of the dosage does not greatly change the result. Toward the 11th month practically no animal respond to 0.5 gm. but even in this period great elevation of dosage shows a definite effect, for almost a third of such animals respond to 4.0 gm. of wheat germ oil.

3. After the 15th month of life no response can be secured with a twenty-fold increase of dosage. —*Abstract, Wistar Institute Bibliographic Service.*

Fluhman, C. F. Gonadotropic hormone. VII. Influence of length of period of administration of equine hormone. *Proc. Soc. Exper. Biol. & Med.* 41: 313. 1939.

Unlike anterior pituitary and chorionic gonadotropins, a given total dose level of an extract of equine gonadotropic hormone produced approximately the same ovarian changes in 21 to 23 day old rats, irrespective of the duration of injections. The uterine weights, however, increased progressively with the duration of injections.—E. T. Gomez.

Fluhmann, C. F., and K. M. Murphy. Estrogenic and gonadotropic hormones in the blood of climacteric women and castrates. *Am. J. Obst. & Gynec.* 38: 778. 1939.

Using previously described methods the authors find estrogen in the blood of 65 of 76 patients, including surgical or radiation castrates. In many women this estrogen occurs with cyclic variations. The source and significance of this estrogen is unknown. There was no obvious relationship be-

tween the amount or cyclic variations and the intensity of climacteric symptoms in individual patients. The gonadotropic hormone was increased in the climacteric group, and its increase was at least roughly proportional to the occurrence of the climacteric complaints. Therapy which was effective in reducing these complaints failed to exert a dependable reduction in the gonadotropic excretion, whether this was by sedation, irradiation of the pituitary, or administration of estrogens.—E. L. S.

Foote, C. E., and E. Witschi. Effect of sex hormones on the gonads of frog larvae (Rana clamitans). Sex inversion in females; stability in males. *Anat. Rec.* 75: 75. 1939.

The sex of fully differentiated larvae of the green frog was established by exploratory laparotomy, at the start of the hibernating season. The lot consisting of 138 ♀ ♀ and 124 ♂ ♂ was split into experimental and control groups. The present paper deals mainly with a group of thirty-two ♂ ♂ which were injected with estradiol and estradiol 3-benzoate 17-butyrate, but failed to show any marked effects after 4½ months, and a group of thirty-eight ♀ ♀ injected with testosterone propionate which under this treatment transform completely into ♂ ♂. The larvae of the latter group, if killed at the end of the experiment (4½ months), had received a total of 3.5 mg. of testosterone propionate. The series of morphological changes leading from the ovarial to the testicular structure are described, and the mode of hormone action is briefly discussed.—*Abstract, Wistar Institute Bibliographic Service.*

Forbes, T. R. Studies on the reproductive system of the alligator. V. The effects of injections of testosterone propionate in immature alligators. *Anat. Rec.* 75: 51. 1939.

Each of 7 ♂ and 6 ♀ alligators received a total of 10 mg. of testosterone propionate by intraperitoneal injection over a period of 11 weeks. The experimental animals and the 9 ♂ and 7 ♀ control animals were approximately 17 months of age when injections began. The oviducts of the injected ♀ ♀ showed a uniform and marked development and growth, and the penes and clitorides of injected animals of both sexes were strikingly hypertrophied. The mesonephroi and mesonephric ducts were not affected in either sex. Histological findings suggested some influence of the hormone on the gonads, but the evidence was not conclusive.—*Abstract, Wistar Institute Bibliographic Service.*

Foss, G. L. Clinical administration of androgens. *Lancet* 236: 502. 1939.

In a male post-pubertal eunuch aged 38, various androgens were given by different routes to maintain sexual function over the period of 1 year. Bland injections of sesame oil were given as controls. A minimum maintenance dose of 40 mg. per week, subcutaneously, was required to maintain sexual function. By inunction, 2 to 3 times this dose was required to produce an equivalent effect, 6 times as much when applied as a tincture, and 20 times the subcutaneous dose when administered by mouth. Progesterone given in conjunction with testosterone propionate acted as a synergist, and prolonged the action of the androgen. Total testicular extracts had little effect when given by injection, and none when given by mouth.—L. B. S.

Gaarenstroom, J. H. Action of sex hormones on the development of the müllerian duct of the chick embryo. *J. Exper. Zool.* 82: 31. 1939.

Injections were made during the 1st days of incubation with autopsy in most cases on the 18th day. Serial transverse sections of 7-day specimens also were examined. Feminization of the gonads of genetic ♂ embryos induced by 100γ or more estradiol benzoate was accompanied by persistence of the left müllerian duct. Injection of 1000γ resulted in persistence of the right müllerian duct in both sexes. Estradiol benzoate caused marked swelling of the müllerian duct. Estrone, injected at beginning of incubation, inhibited for a short period the growth in length of the müllerian duct. Injection of 1500γ androsterone or dehydroandrosterone prevented müllerian ducts from originating in nearly all embryos. This effect was not counteracted when 500γ estradiol benzoate were injected simultaneously.—H. O. H.

Geist, S. H., and U. J. Salmon. The evaluation of the human vaginal smear in relationship to the histology of the vaginal mucosa. *Am. J. Obst. & Gynec.* 38: 392. 1939.

In 60 women with climacteric symptoms, vaginal smears stained with fuchsin, were compared with biopsies of vaginal mucosa. The degrees of atrophy and estrogen deficiency were graded in 4 stages from normal to most marked atrophy. The atrophy and the responses to therapy with estrogen were not always uniform in different areas examined at the same time. The simplicity of the fuchsin staining technique led the authors to recommend this method for rapid clinical control. —E. L. S.

Giedosz, B. Synergistic action of vitamin C and gonadotropic substance (Über die Aktivitätssteigerung der Gonadotropen Substanz durch Ascorbinsäure) *Klin. Wchnschr.* 18: 63. 1939.

The injection (subcutaneous and intravenous) of a total of 1050 mg. of ascorbic acid into rabbits weighing 1500 to 2600 grams augmented the response to subsequently injected prolan (subcutaneous over a 4-day period) or raw pregnancy urine (10 cc. intravenously). The ovaries of rabbits receiving both ascorbic acid and gonadotropin were somewhat larger and contained more corpora

lutea and corpora hemorrhagica than rabbits receiving only the gonadotropic material. The vitamin injections alone had no discernible effect on the rabbit ovary. The numbers of experimental animals were not given, and it was impossible to tell from the data presented whether or not the ovaries of animals receiving both vitamin and prolan were significantly different from the ovaries of the control groups.—M. H. F.

GLASER, E., AND F. RAUFTL. The bitterling test applied to androgens and estrogens (Die Bitterlingsteste auf männliche und weibliche Sexualhormone) *Klin. Wchnschr.* 17: 1120. 1938.

The intraperitoneal injection of 1 cc. of saturated aqueous solutions of a series of steroid hormones into female bitterlings led to an increase in ovipositor length in 36 hours. Of the preparations tested dihydroestrone-benzoate was the most potent, progesterone next, and adrosterone and testosterone less potent per γ of injected material. With the intraperitoneal injection of 1 fish unit, the authors confirmed De Wit, that the reaction to androgens and to progesterone was much more rapid than to the estrogenic materials. By way of comparison it is noted that one fish unit is contained in 0.3 mg. of yohimbine hydrochloride. Their present experiments show that the injection of 1 mg. of yohimbine hydrochloride subdivided into 4 doses into castrate mice led to full cornification, so that with this material 1 estrogenic mouse unit is equal to 3.3 fish units. In the female bitterling injections of estrogens and progesterone give additive effects, but in the male bitterling estrone inhibited the usual effects of progesterone. The authors discuss at some length the conflicting statements in the literature regarding results obtained with the bitterling, particularly with respect to the water solubilities of the compounds used and the faster absorption of oily solutions after injection into the female fish.—M. H. F.

GREENE, R. R., MARIE W. BURRILL AND A. C. IVY. Experimental intersexuality: the effect of antenatal androgens on sexual development of female rats. *Am. J. Anat.* 65: 415. 1939.

Varying dosages of testosterone, testosterone propionate, androsterone, dehydroandrosterone, and androstenedione were used for injection in 256 pregnant rats, from which 108 full term litters were obtained. A total of 155 newborn and 59 adult intersexed rats were studied. The embryonic sexual development of these genetic females had been modified in the male direction. The degree of masculinization obtained was correlated with both total dosage of androgen given and with the period of embryonic development during which treatment was administered. Well modified animals had oviducts, uteri, and an upper vagina, combined with epididymides, vasa deferentia, seminal vesicles, prostates, Cowper's glands, and a penis. Similar modifications were obtained with all the various androgens used.—*Abstract, Wistar Institute Bibliographic Service.*

GREENE, R. R., MARIE W. BURRILL AND A. C. IVY. Experimental intersexuality. The paradoxical effects of estrogens on the sexual development of the female rat. *Anat. Rec.* 74: 429. 1939.

Large doses (0.8 to 100 mg.) of estrogens (estradiol and estradiol dipropionate) were administered to pregnant rats. Twenty-one newborn ♀ offspring were sectioned. There was stimulation of certain ♀ structures (uteri and nipples) and inhibition of other ♀ structures (lower vagina and ovarian capsule). In 20 animals there was partial or complete preservation of wolffian ducts. In 1 animal the wolffian duct was complete on 1 side, in another both ducts were complete. In 5 animals seminal vesicle anlage were noted.—*Abstract, Wistar Institute Bibliographic Service.*

GREEP, R. O., F. L. HISAW AND H. L. FEVOLD. A testis stimulating extract of brewer's yeast. *Anat. Rec.* 73: 297. 1939.

Extraction of dried brewer's yeast with 50% pyridine and 33% acetone yielded a water soluble substance with some hypophyseal-like properties. Normal and hypophysectomized ♂ rats treated with this preparation showed pronounced testicular stimulation. The accessory sexual structures were neither enlarged in the normal animals nor sustained in the hypophysectomized series. It was apparent from microscopic study that the principal effect of the yeast extract was an enlargement of seminiferous tubules. Adult ♂♂ treated with the extract for 16 days following removal of the pituitary did not show testicular regression or loss of capacity to form spermatozoa. The accessories were larger at autopsy than those of operated controls but much smaller than those of the unoperated controls, thus indicating partial maintenance of interstitial cell activity. Gonads of immature ♂ pigeons likewise underwent a moderate hypertrophy with yeast extract treatment. Boiling the extract entirely destroyed its testis stimulating property.—*Abstract, Wistar Institute Bibliographic Service.*

GREULICH, W. W., AND H. THOMAS. An x-ray study of male pelves. *Anat. Rec.* 75: 289. 1939.

The shape and dimensions of the superior aperture of the pelvis were determined in sixty-nine adult white ♂ student by Thoms' method of x-ray pelvimetry. Their pelvic indexes ranged from 77.0 to 121.0 the average being 100.5 Only one of the men studied had a pelvic index as low as 80, which is usually considered typical for white males.

In most of these pelves the forepart of the inlet was more rounded and its pubic portion somewhat wider than is suggested by the usual textbook de-

scription. The average posterior sagittal diameter of the inlet of these males was only slightly smaller than that observed in 200 primiparous white women recently studied. The pubic angle and the greater sciatic notches of the males resembled very closely those described as masculine in the textbooks. In the majority of the male pelves the ischial spines were distinctly heavier than those of the females and they projected farther into the pelvic cavity. There were, however, a few cases in which the spines were not so prominent in men who were apparently quite as masculine as the others. Pelvic roentgenograms of two extremely hypogonadal males disclosed, in one case, sciatic notches, in the other, ischial spines, and in both, superior pelvic apertures which were indistinguishable in size and shape from those of many apparently normal men.—*Abstract, Wistar Institute Bibliographic Service.*

HALL, G. J. Gonadotropic hormone of pregnant mare's serum. *California & West. Med.* 51: 159. 1939.

This is a brief review of the historical and experimental data on equine gonadotropic hormone. In a series of 135 cases receiving the hormone, there were 57.6% of cures in patients with menstrual disturbances. Forty-seven per cent of those with congenital gonadal hypoplasia were cured and 55.8% who were treated for sterility became pregnant. Equine gonadotropic hormone is sufficiently free from serum protein for use in the human, and is the most valuable gonadotropic hormone thus far available for the treatment of menstrual disorders and functional sterility in the female.—I. B.

HARVEY, ETHEL B. An hermaphrodite Arbacia. *Biol. Bull.* 77: 74. 1939.

Of the many thousands of Arbacia opened, only one hermaphrodite was observed. Of the five gonads, four were predominantly female and one male. Fertilization took place when the sperm and eggs were liberated. Development of the eggs was normal. Eggs and sperm functioned normally with other eggs and sperm.—E. L.

HAWKINSON, L. F. Ovarian hypofunction previous to climacteric: report of 300 cases treated with estrogen. *West. J. Surg.* 47: 584. 1939.

The patients ranged from 14 to 35 years (average 27), with average duration of symptoms of 14 months, and in more than ⅓ dating to pregnancy or abortion, and in 12% to salpingectomy or removal of 1 ovary. Symptoms, as in the menopausal syndrome, were largely subjective and due to estrogen deficiency. Of the 265 who had definite menstrual disturbances, 174 showed scanty menstruation. Standardized estrogenic preparations, in varied quantity, were used. Some responded to oral therapy alone, which was tried first in each instance; others required hypodermic administration of as much as 10,000 I.U. thrice weekly. Combined oral and hypodermic treatment proved advantageous in several cases. Doses were gradually reduced with appearance of satisfactory responses. The average patient required treatment for 3 to 4 months. There were no ill effects, with exception of an occasional local reaction at site of injection. The majority of symptoms were alleviated in 44.3%, 31% were improved and 24.7% experienced little or no relief. Forty-four patients (74.5%) found that estrogen treatment decreased the severity and frequency of attack of menstrual migraine. Fourteen (31.8%) obtained complete relief from headache. In 54 patients with low Hb, 29 (57.4%) showed improvement in Hb level, although the majority had experienced difficulty in maintaining an improved level with liver and iron therapy.—H. O. H.

HENSEL, G. The corpus luteum hormone and hair growth: an experimental study (Corpus luteum-Hormon und Haarwachstum: eine experimentelle Studie). *Ztschr. f. d. ges. exper. Med.* 104: 182. 1938.

Normally, local depilation in the non-pregnant guinea pig was followed within a few days by evidence of regrowth of hair. In the latter part of pregnancy, however, indications of regrowth were not evident, but appeared some time after termination of pregnancy. Repeated injections of corpus luteum hormone (lutren) in non-pregnant guinea pigs prevented the growth of hair in depilated areas. When hormone administration was stopped, growth again took place. Follicular hormone had no effect in arresting the growth of hair.—A. E. Hansen.

HOHLWEG, W., AND H. H. INHOFFEN. Pregneninolon, a new orally active preparation of corpus luteum hormone (Pregneninolon, eine neues per os wirksames Corpus luteum-Hormon-Präparat). *Klin. Wchnschr.* 18: 77. 1939.

The injection of a total of 2 mg. of pregneninolon over a period of 5 days produced full progestational proliferation in castrate rabbits, so that this compound when injected possesses about ½ the potency of progesterone. However, when given orally a total of 4 mg. was equally effective orally over the same period of time, which was in sharp contrast to progesterone, which was ineffective by mouth even in 60 times the subcutaneous unit. Similar to progesterone this new compounds was effective in producing uterine proliferation when applied to the skin in doses of 10 mg.—M. H. F.

HOWARD, EVELYN. Effects of castration on the seminal vesicles as influenced by age, considered in relation to the degree of development of the adrenal X zone. *Am. J. Anat.* 65: 105. 1939.

The X zone is a transitory differentiation of the adrenal cortex characterized in mice by certain responses to sex hormones. There were marked differences in the condition of the seminal vesicles

in mice following castration, depending upon age at operation, and related to degree of development of the adrenal X zone. Thus in animals castrated while the vesicles were still in a rudimentary state, there ensued a marked subsequent differentiation of the vesicles, with development of alveolar structure and columnar epithelium. This was associated with hypertrophy of the X zone. If animals were castrated after columnar epithelium had differentiated but before secretory activity had started, the vesicles were maintained with little or no evidence of degeneration for 40 to 100 days, while the X zone remained hypertrophied. In animals castrated after sexual maturity, when the primary X zone had disappeared, the vesicles underwent a degeneration which remained incomplete during the period in which the secondary X zone differentiated in the adrenal cortex. One hundred days after castraction the secondary X zone had frequently disappeared from the cortex and in these cases the vesicles were in a state of marked and uniform degeneration, with cuboidal epithelium. The most probable explanation for these findings is that the adrenal X zone exerts andromimetic activity to a greater degree than does the permanent cortex.—*Abstract, Wistar Institute Bibliographic Service.*

Howard, Evelyn. A note on the reactions of the mouse uterus to ovariectomy in the presence of the x-zone. *Bull. Johns Hopkins Hosp.* 65: 341. 1939.

The degree of uterine involution after ovariectomy was the same regardless of the presence or absence of an x-zone in the adrenal. This was further evidence for the view that the x-zone exerts no androgenic function (Gersh and Grollman). Contrary to the findings of Parkes and Brambell the reaction of the uterus of the mouse to ovariectomy was not found to differ from that which occurs in the rat.—A. G.

Howard, J. E. and S. A. Vest, Jr. Clinical experiments with male sex hormones. II. Further observations on testosterone propionate in adult hypogonadism and preliminary report on the implantation of testosterone. *Am. J. M. Sc.* 198: 823. 1939.

Observations extending over a period of 2 years on the effects of testosterone propionate in sesame oil given by hypodermic injections to 22 adult patients suffering from hypogonadism are reported. Development or reestablishment of secondary sexual characteristics with induction of normal libido and potentia have been observed. The psychological effects were excellent. Two doses per week of 25 mg. each has seemed adequate. Smaller doses, if given more frequently, seemed equally effective, but if injections were given only twice weekly smaller doses than 25 mg. proved inadequate for good therapeutic results. A preliminary report is given on the surgical implantation of compressed pellets of pure testosterone. Results thus far are encouraging and have seemed equally efficacious as those resulting from injections, far less troublesome to the patients, and somewhat sparing of material.—R. G. H.

Jett-Jackson, Cecelia E. A study of the hormonal factor in the repair of uterine tissue in the albino rat. *Anat. Rec.* 74: 79. 1939.

Thirty-seven virgin albino rats were divided into 4 main groups: normals, castrates, castrates receiving estrin, castrates receiving progesterone. In each instance the uteri were slit longitudinally. After an interval of 5, 9, or 14 days, the tissue was removed and sections were made through the area of repair. Microscopic study revealed little discernible hormonal effect (e.g., acceleration or retardation of repair) at any of the 3 periods. Sections from animals injected with colchicine revealed mitosis of smooth muscle cells. Complete repair, with disappearance of the scar, was found at 21 to 25 days.—*Abstract, Wistar Institute Biographic Service.*

Karnaky, K. J. Clinical use of the new synthetic estrogenic hormone, stilboestrol: preliminary report. *South. M. J.* 32: 813. 1939.

This new synthetic product differs radically from the estrogens in its chemical structure. It was used to treat 189 cases. These included amenorrhea, hypomenorrhea, meno-metorrhagia, patients suffering from disturbances at the menopause, and normals. The drug was equally effective by mouth or intramuscularly, and was tolerated in doses up to 55 mgs. intramuscularly without signs of toxicity. It was palatable. Occasionally it produced nausea, but this could usually be avoided by selecting the time of administration. On bases of the cases studied, it was found that diethyl stilboestrol was as effective as the natural estrogens and could be substituted for them.—J. C. D.

Kingsbury, B. F. Atresia and the interstitial cells of the ovary. *Am. J. Anat.* 65: 333. 1939.

There is reviewed the interpretation of the interstitial cells as endocrine cells of the ovary. From a detailed study of their origin from the theca of atretic follicles in the cat, it was concluded that there is no evidence of their glandular nature. On the contrary it is suggested that the content of lipid droplets characteristic of such cells expresses an alteration in the metabolism of the stroma cells determining a retention of lipid substances. The relation of follicular epithelium and theca stroma cells, the causation of atresia and the source of ovarian hormones are briefly discussed.—*Abstract, Wistar Institute Bibliographic Service.*

Kirschbaum, A., C. A. Pfeiffer, Jean D. Van Heuverswyn and W. U. Gardner. Studies on gonad-hypophyseal relationship and cyclic

osseous changes in the English Sparrow, Passer domesticus L. *Anat. Rec.* 75: 249. 1939.

Seasonal hyperossification of the skeletal system of the ♀ sparrow was correlated with seasonal ovarian activity. Although the bone changes have not thus far been duplicated with injections of estrogens, complete activation of the ovary during the non-breeding season was accompanied by the laying down of osseous spiculaes in the marrow cavities. Juvenal sparrow testes grafted into ♀ ♀ exposed to added daily light ration in the winter were stimulated to produce spermatozoa. Gonadotropic secretion of the ♀ hypophysis failed to stimulate the hosts' own ovaries, whereas the grafted testes were fully stimulated. The testis was more responsive than the ovary to gonadotropic secretion. The sparrow ovary secretes ♂ hormone as indicated by darkening of the ♀ bill during the normal breeding season and experimentally when the ovary was stimulated to full activity out of season.—*Abstract, Wistar Institute Bibliographic Service.*

Lafitte, A., and G. Huret, Ovarian disequilibrium and dermatoses. The therapeutic action of the male sex hormone in certain types of psoriasis and eczema (Déséquilibre ovarien et dermatoses. Action thérapeutic de l'hormone mâle dans certain variétés de psoriasis et d'eczema). *Presse méd.* 47: 472. 1939.

A relationship between menstruation and eczema was noted in some patients. Estrogens were usually ineffective in treatment, but testosterone propionate caused regression of symptoms without, however, a permanent cure. The dose was 10 mg. on alternate days, given 5 times from the 12 to the 20th day of the cycle.—A. E. M.

Lane, C. E., and R. F. Davis. The ovary of the adult rat. I. Changes in growth of the follicle and in volume and mitotic activity of the granulosa and theca during the estrous cycle. *Anat. Rec.* 73: 429. 1939.

Based upon total volume determinations of granulosa, theca and antrum, with correlation of volume changes with estrous cycle phases, the conclusions were drawn that: (1) during diestrus the ovary is subject to increased FSH stimulation; (2) it is unlikely that the slight increase in large follicles occurring during proestrus and estrus is sufficient to account for estrous phenomena; (3) follicles less than 200 u in diameter are inactive mitotically and are thought to be physiological quiescent; (4) between 200 and 300 μ diameter, the follicles in any stage of the cycle show mitotic activity in granulosa and theca which is significantly augmented—such follicles are thought to be beginning their journey toward ovulation or atretic degeneration; (5) mitotic index of the ovary as a whole is highest in metestrus; (6) maximum total granulosa volume is seen in diestrus; (7) maxima in the curves of total antrum and total volume of theca occur in proestrus.—*Abstract, Wistar Institute Bibliographic Service.*

Lipprose, O. Results obtained by treatment with male sex hormone (Resultados del tratamiento con hormonas sexuales masculinas). *Semana méd.* 46: 911. 1939.

Treatment with testoviron in two cases of mild diabetes brought about a reduction of the blood sugar to normal values whereas a case of moderately high glycemia showed considerable improvement, It was found that the contractive power of the urinary bladder was greatly enhanced and the contractility of the striped muscles stimulated.—A. E. M.

Loesser, A. The Pharmacology and toxicology of 4, 4′ dioxy-a, b, diothylstilbestrol (Zur Pharmokologie und Toxikologie des 4, 4′ a, b,-Diäthylstilbens), *Klin. Wchnschr.* 18: 346. 1939.

The daily subcutaneous injection of 1 mg. of the diethylstilbestrol into rats led to bleeding from the gut and vagina, in contrast to the results with similar quantities of estrone, which produced bleeding in the dog but in no other mammal. Injected into the uterus, stilbestrol led to local uterine growth but no effect on the thyroid, which was also in contrast to the action of estrone. Animals injected with the stilbestrol showed hypertrophy of the adrenals with loss of adrenal fat. When fed, instead of injected, at levels of 1 to 5 mg. daily, there was in addition hemorrhage in the adrenal cortex. Daily injection of 1 mg. stilbesterol for 21 days led to fatty infiltration of the liver with hemosiderin deposits in the reticuloendothelial system. The kidneys of such animals were loaded with heavy deposits of icteric pigments and blood bilirubin and urinary urobilinogen were markedly increased in injected animals.—M. H. F.

McCahey, J. F., and A. E. Rakoff. An estrogenic property of testosterone propionate. *J. Urol.* 42: 372. 1938.

Twenty-four young adult spayed mice were given testosterone propionate in doses varying from 0.005 to 10 mgs. Injections were begun 7 days after ovariectomy and the animals were killed 4 days after the first injection. With multiple doses, a minimum total of 0.1 mg. produced vaginal cornification and ·2 mg. uterine estrous. From this the authors conclude that: "The demonstration that testosterone propionate has estrogenic properties shows that ovarian follicular hormone is not the only substance which can cause a positive Allen-Doisy reaction. Positive Allen-Doisy tests secured with extracts of tissues or excretions of males are, therefore, insufficient basis for the contention that a female sex endo crine is normal to males and that such an element may be a factor in disease.—J. C. D.

MARTINS, T., J. R. VALLE AND A. PORTO. The endocrine control of the motility of the male accessory genital organs. The comparative pharmacology in vitro of the spermatic cord and seminal vesicles of normal, castrated and hormone treated Rhesus monkeys (Die endokrin Kontrolle der Motilität der männlichen akzessorischen Genitalorgane. Vergleichende Pharmakologie in vitro der Samenleiter und Samenblasen von normalen, kastrierten und mit Sexualhormonen behandelten Rhesusaffen). *Arch. f. d. ges. Physiol.* 242: 155. 1939.

Spontaneous contractions of the spermatic cord and seminal vesicles were observed only in castrated monkeys previously treated with estradiol benzoate. Adrenalin, acetylcholin and nicotine stimulated contraction in these organs under all conditions. $BaCl_2$, ephedrin, histamine, hydrastinine and yohimbine stimulated only organs removed from castrates or animals previously injected with estradiol benzoate.—A. G.

MATTHEWS, S. A. The effects of light and temperature on the male sexual cycle in Fundulus. *Biol. Bull.* 77: 92. 1939.

Light was not essential for complete activation of the testis but low temperatures had a retarding influence on sperm maturation.—E. L.

MAZER, C., AND S. L. ISRAEL. The effect of crystalline corpus luteum hormone, progesterone, on the ovaries and related endocrine organs. *Am. J. Obst. & Gynec.* 38: 625. 1939.

In doses of 0.5 mg. thrice weekly for 9 weeks progesterone produced no inhibition of ovulation or follicle growth or luteinization. There were no changes in cycles, or in pituitary, ovarian or adrenal weights. Larger doses produced inhibition of ovarian functions.—E. L. S.

MÜHLBOCK, O. Free and combined oestrogens in the blood during pregnancy. *Lancet* 236: 634. 1939.

Using the extraction method of Dingemanse, the retroplacental blood samples of pregnant women at childbirth showed that the estrogenic hormone was present 30 to 50% in the combined form. Therefore in order to obtain the total estrogenic hormone content it was found necessary to examine the blood after hydrolysis.—L. B. S.

NELSON, W. O., AND C. E. MERCKEL. Maintenance of spermatogenesis in hypophysectomized mice with androgenic substances. *Proc. Soc. Exper. Biol. & Med.* 38: 737. 1938.

Androgenic substances, including androstanedione, androstanediole, testosterone and testosterone propionate prevented degenerative changes of the seminiferous tubules and spermatogenesis maintained in hypophysectomized mice when administered in amounts ranging from 0.4 to 1.0 mg. daily beginning on the 2nd day after hypophysectomy. Of the androgens used, androstanedione proved most effective. Degenerative changes of interstitial cells, adrenals and thyroids were not prevented by the treatment.—E. T. Gomez.

PERCY, C. J. Influence of diet on gonad activity of English sparrow (Passer domesticus, L.) *Proc. Soc. Exper. Biol. & Med.* 38: 716. 1938.

Wheat seeds exposed to Spermi Ultraviolet light for 24 to 300 hours and fed to juvenal ♂ and ♀ sparrows during the months of Dec., Jan., and Feb., stimulated pigmentation of beaks of both sexes, spermatogenesis and functional activity of the sperm ducts in ♂ ♂, and in ♀ ♀ development of follicles and oviduct comparable to that found in laying birds. This response was similar to that produced by the injection of prehypophyseal extract.—E. T. Gomez.

PETIT, G., P. GLEY AND E. BERAUT. Experimental production of prostatic adenoma (Réalization expérimental de l'adénome prostatique). *Bull. Acad. de méd.* 121: 430. 1939.

Immature dogs were treated with 1000 U of estradiol benzoate daily. Prostates as well as prostatic urethrae showed evidence of stimulation. The prostates acquired finally the size and structure of that of the adult, but in no instance was an adenoma found. Urethrae showed proliferation of the epithelium, formation of new glands and development of a structure possessing all characteristics of adenoma.—A. E. M.

PINTO, A. The action of folliculin and of testo sterone in labor of childbirth (Acción de la foliculina y testosterona en el trabajo de parto). *Semana méd.* 45: 1000. 1938.

Injections of 10,000 U of estrone caused an increase in frequency and intensity of the uterine contractions and induced regularity of rhythm. A favorable influence on dilatation of the cervix was observed. There was no unfavorable effect upon the fetus. Testosterone, given in dosage of 10,000 U, did not have the slightest influence upon labor. —A. E. M.

POYNTER, HELEN. Testis hormone secretion in the rat under conditions of vasectomy or isolation. *Anat. Rec.* 74: 355. 1939.

Histology and weight of testes, seminal vesicles, and prostate of 117 rats vasectomized at ages varying from 15 days to 15 months and allowed to survive for 20 days to 6 months did not vary significantly from those of 99 normal littermate controls. Since seminal vesicle weight in the rat varies in direct relation to the amount of testis hormone present, vasectomy did not modify the rate of male hormone secretion. Male rats isolated from females from 1 month of age to 7 to 10 months of age failed to show significant variation from non-isolated littermate controls.—*Abstract, Wistar Institute Bibliographic Service.*

RAKOFF, A. E. Studies on the concentrations of estrogenic and gonadotropic hormones in the serum of pregnant women. *Am. J. Obst. & Gynec.* 38: 371. 1939.

The hormones were determined by injection of unextracted serum in divided doses into spayed or immature mice, indicating that much hormone is lost when extracted serum has been used. Titration was done on 162 samples in 40 pregnancies, establishing the normal high peak of gonadotropic material by the end of the first trimester, and the maintenance of a low level from the 20th week on. Serum estrogen increased slowly, and then more rapidly toward term. In 22 toxemias the late prolan values were high, the estrin values low. This high prolan finding occurred a few weeks before symptoms in 3 cases where data were available. In nephritic toxemia 4 cases indicate a similar disturbance in hormone balance. It is believed decreasing serum prolan estimations give a better means of detecting fetal death in utero than the Friedman test.—E. L. S.

REYNOLDS, S. R. M., AND F. I. FOSTER. Peripheral vascular action of estrogen in the human male. *J. Clin. Investigation* 18: 649. 1939.

The effect of intramuscular injection of estrogens upon finger volume and skin temperature in 20 adult males was a prompt increase in volume in two thirds, without change in temperature. The change persisted for more than 2 hours. Since there appeared to be no alteration in rate of blood flow, the effect is thought to depend on the small vessels beyond arterioles.—E. L. S.

RODEWALD, WILHELMINE, The antigonadotropic effect of carinoma serum (Die Antigonadotrope Wirkung von Carcinomserum). *Klin. Wchnschr.* 18: 26. 1939.

Serum from various patients was injected into infantile mice in doses of 0.25 cc. twice daily for two days, after which the mice received a total of "1 unit" of anterior pituitary preparation in six divided doses over 48 hours. At autopsy 100 hours after the 1st hormone injection the ovaries were compared with those of controls receiving only the serum or only the pituitary. Serum of all kinds when injected alone had either no effect or a slight stimulating effect. Serum from cases of carcinoma inhibited the action of the pituitary extract while sera from a variety of other types of patients had an augmenting effect upon the ovarian reaction. Other work was cited to indicate that this effect of carcinoma serum upon the gonadotropic activity was not specific in that such sera had already been shown to inhibit the thyrotropic and melanophore reactions.—M. H. F.

RUBENSTEIN, H. S., A. R. ABARBANEL AND D. N. NADER. Influence of testosterone propionate on vaginal opening in the immature albino rat. *Proc. Soc. Exper. Biol. & Med.* 39: 20. 1938.

Daily injections of 1 mg. testosterone propionate in castrate and normal ♀ rats beginning at the 26th day of life stimulated vaginal opening in 10 to 11 days.—E. T. Gomez.

SALABER, J. A., AND E. B. DEL CASTILLO. The action of ethinylestradiol, a derivative of follicular hormone (Acción del etinilestradiol, derivado de la hormona folicular, por via oral). *Semana méd.* 46: 6. 1939.

Three patients, aged 21, 31 and 31 years, who suffered from secondary amenorrhea without other evidence of endocrine disturbance were treated with oral doses of ethinylestradiol, 0.3 mg. daily, 7.4 to 15 mg. total. Menstruation occurred 4 to 6 days after cessation of treatment and lasted for 4 to 7 days.—A. E. M.

SELYE, H. The effect of progesterone on the mouse ovary as influenced by gestation. *Anat. Rec.* 75: 59. 1939.

Experiments on the adult mouse indicated that daily administration of 1 mg. of progesterone for 5 days sufficed to cause corpus luteum involution and marked ovarian atrophy. In pregnant mice, even 8 daily injections of 1 mg. of progesterone did not cause significant ovarian atrophy or any detectable change in the corpora lutea of gestation. Since hypophysectomy likewise caused rapid involution of the corpora lutea in the non-pregnant mouse but remains without detectable influence on the corpora lutea of gestation, it seemed possible that the ovarian atrophy caused by progesterone was due to an inhibition of the gonadotropic function of the hypophysis. It was concluded also that the gonadotropic hormone of the placenta which maintains the corpora lutea of gestation is not inhibited by progesterone.

Delivery and the onset of lactation were not prevented by massive doses of progesterone. This finding was not in accordance with the assumption that discontinuation of corpus luteum hormone production is the cause of delivery at term and of the initiation of milk secretion.—*Abstract, Wistar Institute Bibliographic Service.*

SHORR, E., F. H. ROBINSON AND G. N. PAPANICOLAOU. A clinical study of the synthetic estrogen stilbestrol. *J. A. M. A.* 113: 2312. 1939.

In a series of 44 cases showing predominantly the menopausal syndrome, stilbestrol produced toxic symptoms of central origin in 35 (80%), evidenced by nausea, vomiting, anorexia, diarrhea, lassitude, acute psychotic reactions and cutaneous rashes, which occurred whether the material was injected or given orally. It was actively estrogenic, inducing follicular smears, vaginal cornification, and withdrawal bleeding in amenorrhea and the menopause, and relieved the subjective symptoms of the latter. No relation was found between size of dose and development of toxic reactions, nor was evidence obtained of an ac-

quired tolerance. While less active than estradiol benzoate parenterally, it proved more effective by mouth, losing but ½ or less of its activity. It was less active than ethinyl estradiol by mouth. The oral estrogenic unit for human use lay between 2 and 4 mg. The authors urge extreme caution in its clinical use until the nature of the side effects are understood.—H. O. H.

SOUTHWICK, W. E. Activity-preventing and egg-sea-water neutralizing substances from spermatozoa of Echinometra subangularis. *Biol. Bull.* 77: 147. 1939.

Dry sperm of the reef urchin is active for only 50 to 120 seconds after removal from the testes. But the addition of sea water may reactivate the spermatoza for 6½ hours. An activity-preventing substance accumulates in sperm suspensions and this substance may be identical with the substance neutralizing the agglutinating power of egg-sea-water.—E. L.

SOUTHWICK, W. E. The agglutination phenomenon with spermatazoa of Chiton tuberculatus. *Biol. Bull.* 77: 157. 1939.

Dry sperm of Chiton tuberculatus, introduced into the mantle cavity of a male or an immature female, are clumped, the points of attachment being the tail. Clumping reaction is due to a sticky substance on the tail. This reaction can be prevented by saponin, sodium taurocholate and trypsin.—E. L.

STARKEY, W. F., AND J. H. LEATHEM. Action of estrone on sexual organs of immature male cats. *Anat. Rec.* 75: 85. 1939.

Estrone was injected intramuscularly, over varying periods of from 10 to 30 days, into 10 immature ♂ cats. Seven ♂ ♂, 6 of which were litter mates of the experimental animals, served as controls. All experimental animals showed hypertrophy of the glandular epithelium of the prostate. In addition, there resulted metaplasia of the urethral and ductal epithelium without leucocytic infiltration. No evidence of atrophy was observed in the reproductive tract of the experimental animals.—*Abstract, Wistar Institute Bibliographic Service.*

STEHLE, F. The significance of the reaction 1 to prolan in the ovaries of the infantile white mouse (Ueber die Bedeutung der HVR 1 im Ovarium der infantilen weissen Maus). *Zentralbl. f. Gynäk.* 63: 2119. 1939.

From a large laboratory service the author selected the 156 negative Aschheim-Zondek tests where there were no endocrine anomalies suspected in the women. Of these 21% had a positive reaction type 1, i.e. with mature follicles in the ovaries of the immature mice. Therefore the reaction cannot be taken to have any significance in diagnosing pregnancy.—E. L. S.

STEIN, I. F., AND M. R. COHEN. Surgical treatment of bilateral polycystic ovaries' amenorrhea and sterility. *Am. J. Obst. & Gynec.* 38: 465. 1939.

Following up an earlier report on 7 women, the authors bring the total to 28 who have been treated by a wedge resection of both ovaries. The results have been marked improvement of menstrual rhythm in 25 of these women. Of 19 married women 11 had 15 pregnancies. There have been no recurrences to date. There is speculation without data about the endocrine mechanism involved.—E. L. S.

TAPELLA, P. A. Precordial pain in ovarian dysfunction (Dolor precordial en las disfunciones ováricas). *Semana méd.* 46: 275. 1939.

The pain was felt between the heart and the base of the sternum, sharp, piercing and nonradiating. It appeared generally 8 to 12 days preceding menstruation, lasted for 3 to 5 days and disappeared with menstruation. The patients were less than 35 years of age and usually had a history of genital pathology, predominantly ovarian dysfunction. Cardiac disease was absent. Change or suppression of ovarian function (pregnancy, castration) caused subsidence of the pain. Progesterone treatment was beneficial.—A. E. M.

TAYLOR, H. C., R. C. WARNER AND C. A. WELSH. The relationship of the estrogens and other placental hormones to sodium and potassium balance at the end of pregnancy and in the puerperium. *Am. J. Obst. & Gynec.* 38: 748. 1939.

The sodium and potassium balance is positive before delivery, and becomes negative a few days post partum, after the drop in excretion of estrogen, pregnandiol, and gonadotropic hormone. Results in a few cases with toxemias are interesting, in that sodium was lost in spite of maintained high prolan, but with diminished estrogen and pregnandiol output. The therapeutic administration of estradiol prolonged the positive sodium balance beyond the early puerperium. It seems obvious that there is a parallelism between high amounts of estrogens, possibly also progesterone, and sodium retention. The potassium retention is not so clearly outlined. Causal relations are not proved. The association of endocrine changes, sodium and water retention, edema, and toxemias is stressed. There are 3 normal cases, one preeclamptic, one with fetal death, and one normal with estrogen trearment.—E. L. S.

THALEN, M. H. G. A., AND L. A. M. STOLTE. Progestin in repeated abortion. *Nederl. tijdschr. v. geneesk.* 31: 3880. 1939.

The clinical histories of 9 females in whom pregnancy usually terminated in abortion were reviewed, 29 pregnancies having produced only 2 living children. Following progesterone treatment, 12 pregnancies resulted in 8 living births,

although it was admitted that in all instances the result was probably not ascribable to progesterone. However, it was believed that progesterone is a valuable addition in treatment of habitual abortion.—*From J.A.M.A. Abstracts.*

THOMPSON, W. O., AND N. J. HECKEL. Male sex hormone: clinical application. *J. A. M. A.* 113: 2124. 1939.

On the basis of their own and of observations made elsewhere the authors concluded that testosterone propionate is useful in substitutional therapy as contrasted to stimulation therapy of gonadotropic material, and hence is indicated in the treatment of castrate and eunuchoid persons. It was found to cause marked reduction in spermatozoa count in normal ♂♂, and did not appear effective in treatment of benign prostatic hypertrophy. Its effectiveness in treatment of senile changes remains to be determined.—H. O. H.

TISLOWITZ, R. The action of estrogens in inducing mitoses in the muscle, connective tissue and epithelium of the prostate and seminal vesicle of mice as determined by the colchicine technique. *Anat. Rec.* 75: 265. 1939.

The effect of estrogens in causing hyperplasia in the fibromuscular stroma of the prostate and seminal vesicle of castrated animals was readily observed by the aid of colchicine. There resulted also to some extent mitoses in the epithelium induced by estrogens.

The difficulty inherent in testing a mixture of androgens and estrogens was pointed out and a method for distinguishing qualitatively between the effect of these substances was suggested.

An artificial estrogen, diethylstilboestrol, produced changes in the prostate and seminal vesicle, similar to those caused by estrogens with a steroid structure.—*Abstract, Wistar Institute Bibliographic Service.*

TRABUCCO, A., AND G. A. TABOSSI. Hormone treatment of adenoma of the prostate (Algunas consideraciones clinicas acerca del tratamiento hormonal del adenoma de la próstata). *Semana méd.* 46: 39. 1939.

Testosterone propionate brought about improvement in subjective and relief of pollakiuria. First grade prostatitis was benefited by treatment, and and 3rd grade prostatis was not influenced. Continual medication was found necessary, with adjustment to the individuality of the patient.—A. E. M.

TRONCI, L. The influence of folliculin on the fasting ketonuria in normal and castrate rats (Influenza della follicolina sulla chetonuria da digiuno di ratte normali e castrate). *Sperimentale* 92: 594. 1938.

The ketonuria of starvation in the normal rat was increased by folliculin but no influence was seen on the slight ketonuria caused by starvation in the castrate animal.—A. E. M.

TURNER, C. D. The modification of sexual differentiation in genetic female mice by the prenatal administration of testosterone propionate. *J. Morphol.* 65: 353. 1939.

Prenatal administration of testosterone propionate induced persistence of wolffian duct derivatives and inhibited formation of the caudal vagina. Persistence of müllerian ducts was not induced in genetic males nor were they inhibited in genetic females. Determination of genital primordia progressed from anterior to posterior. Prenatal androgen treatment did not induce hypertrophy of the ovarian medulla nor inhibition of ovarian cortex formation; treatment did, however, impair ovarian function as reflected in failure of adult intersexed mice to display normal vaginal estrous periods. Ovulation and luteinization did not occur in well-affected animals. Ovarian follicles became atretic and interfollicular areas were eventually occupied by epithelioid cords. A full complex of wolffian duct derivatives persisted when the androgen was administered between the 10th and 13th days of gestation. After the 15th day, administration of androgen did not induce persistence of wolffian duct derivatives. Male sex hormone administered on the 13th and 14th days of gestation had a tendency to induce unilateral persistence of wolffian duct derivatives or persistence of cranial and caudal remnants only. Masculinization of the urogenital sinus and genital tubercle was induced by treatment during terminal stages of gestation. —*Abstract, Wistar Institute Bibliographic Service.*

UOTILA, U. U. The masculinizing effect of some gonadotropic hormones on pullets compared with spontaneous ovariogenic virilism in hens. *Anat. Rec.* 74: 165. 1939.

In immature pullets F.S.H. stimulated the medullary cord cells, and, to a lesser degree, interstitial cells, resulting in masculinization of head furnishings. F.S.H. also stimulated the interfollicular cells, with hypertrophy of oviducts. L.H. stimulated principally interstitial cells of the ovarian medulla, producing masculinization of head furnishings. P.M.S. hormone combined the effects of L.H. and F.S.H. P.U. hormone stimulated the interstitial cells very feebly. Androsterone caused slight cortical atrophy and masculinization of head furnishings. Estrin inhibited comb growth to some degree and retarded ovarian cortical development with atrophy of medullary components. F.S.H. and L.H. produced masculinizing ovarian overgrowths which fulfilled several of Meyer's morphological and physiological criteria for 'arrhenoblastomata.' Marked similarities were noted between these experimental overgrowths and spontaneous ovarian tumors. A working hypothesis concerning the genesis of masculinizing ovarian overgrowths was suggested.—*Abstract, Wistar Institute Bibliographic Service.*

VARANGOT, J. Estrogenic activity and toxicity of stilbestrol (4, 4-dihydro-a, B-diethylstilbene) (Activité oestrogene et toxicité du stilboestrol). *Presse méd.* 47:725. 1939.

Estrogenic activity of stilbestrol in animals was comparable to that of the natural estrogens, with the difference that it was equally active when given by mouth. Castrate rats gave a 100% positive vaginal response to doses of 0.1γ, indicating an activity 6 times greater than that of estradiol benzoate. Clinically, satisfactory responses were observed in cases of hot flashes after castration and of senile pruritis vulvae, the latter to dosages of 1 to 2 mg. daily. In treatment of amenorrhea it proved inferior to estradiol benzoate, and withdrawal bleeding was not obtained following dosages of 14 to 28 mg. Mice fed 0.5 mg. per gm. body weight died within 3 to 4 days. Toxic effects frequently appeared in patients after 2 days of medication, with dosages of 0.1 to 1 mg. daily, as shown by nausea, vomiting and vertigo which were occasionally so severe as to necessitate cessation of treatment. The malaise persisted for 2 days following withdrawal of medication. Other patients developed symptoms only after 1 or even 2 months of treatment, and often complained of severe gastric pain in addition to the symptoms mentioned. In view of these experiences, stilbestrol cannot be considered as a substitute for the natural estrogens.—A. E. M.

VENABLE, J. H. Intra-uterine bleeding in the pregnant albino rat. The 'placental sign.' *Anat. Rec.* 74: 273. 1939.

The uterine lumen remains patent mesometrially from time of implantation (6th day) until development of secondary mesometrial attachments occludes the lumen at each embryonic site (11th day). Disappearance of the antimesometrial attachment (15th day) reestablishes the lumen throughout and initiates a period of unipolar mesometrial attachment which persists until term. There are two sources of intra-uterine bleeding: 1) Eroded subepithelial decidual vessels adjacent to the ectoplacental cone give rise to extravasated blood on the 8th day. 2) The distinctive blood sinuses of the giant cell area of the decidua give rise to intra-uterine bleeding on the 11th day. Closure of the uterine lumen at each embryonic site (late on 11th day) prevents subsequent intra-uterine bleeding. Blood appears in the vagina on the 11th and 12th day and is added to each day until the 16th. All blood destined to enter the vagina is derived from the lowest implantation site of each uterine cornu. This extravasate forms a reservoir of ageing blood below the lowest implantation site of each uterine horn, from which there is gradual drainage into the vagina. Blood trapped in each inter-embryonic segment of the uterine lumen is resorbed before the lumen opens (15th day). There is in consequence no increment in vaginal blood at this time.—*Abstract, Wistar Institute Bibliographic Service.*

VIDGOFF, B. The hormonal control of the prostate and its relation to clinical prostatic hypertrophy. Urology. *J. Urol.* 42: 359. 1939.

This is a review and careful summary of the literature with over 100 references.—J. C. D.

WEBSTER, B. The treatment of hypogonadism in the adolescent male. *J. Pediat.* 13: 847. 1938.

The use of testosterone propionate in 6 cases of adolescent hypogonadism is reported. It appeared to bring about striking anatomical changes resulting in the growth of the penis, scrotum and prostate. Secondary sexual characteristics such as hair growth and voice change occurred as in the normal process of puberty. It would seem that this substance offers a means of inducing, in hypogonadal adolescent males, the anatomical changes which normally occur at puberty.

Growth spurts were noted in each case during the period of the administration of testosterone propionate. In the short time during which these patients were under treatment, no x-ray evidence of epiphyseal closure could be detected.—M. B. G.

PARATHYROIDS

VACIRCA, F. Parathyroid, calcium and glutathione (Paratiroide, calcio e glutatione). *Sperimentale, Arch. di biol.* 93: 168. 1939.

Parathyroid hormone produced in the normal dog hypercalcemia but seldom augmented the blood glutathione. Thyroparathyroidectomy caused an immediate drop in blood calcium which was followed by a gradual decrease in glutathione. This could be observed only in animals surviving for a sufficient length of time. While parathyroid hormone promptly raised the calcium level, the glutathione decreased progressively despite the subsidence of parathyroid deficiency symptoms. Alimentatin with milk did not influence either calcium nor glutathione in the normal dog; the injection of calcium salts caused hypercalcemia without affecting the glutathione. An influence of the parathyroids on glutathione metabolism is claimed.—A. E. M.

THYMUS

BOWMAN, J. E., AND C. L. JACKSON. Chronic stridor in infancy. *J. Pediat.* 15: 477. 1939.

The causes of stridorous breathing in 30 consecutive infants chronically affected included 5 with enlarged thymus, 14 with congenital excessive curling and flaccidity of the epiglottis and 3 with other congenital malformations of the larynx.

Diagnosis of enlargement of the thymus gland depended upon finding by lateral x-ray of kinking, narrowing and displacement backward of the trachea by a shadow posterior to the manubrium of the sternum. Pancoast's method of taking roentgenograms is valuable in the diagnosis and evaluation of thymic compression. Before classifying a child in this group, definite improvement in the

symptoms must have appeared before 48 hours after institution of x-ray therapy.—M. B. G.

MITCHELL, A. G., E. BOYD, W. E. CHAMBERLAIN, N. EINHORN, H. F. HELMHOLZ AND A. H. SPOHN. Panel discussion on the thymus gland. *J. Pediat.* 14: 534. 1939.

The chairman, A. G. Mitchell, summarized the discussion as follows:

It is proper to say that the thymus gland occasionally causes symptoms of compression, but other causes for compression symptoms must be excluded before the thymus is incriminated. It is possible that certain enlargements of mediastinal structures can cause symptoms of compression and that radiological treatment has some effect on these symptoms.

Great caution is necessary in stating that roentgenogram shows thymic enlargement. There is no relation between the size of the thymus gland and sudden death or convulsions.

If the thymus is related to sudden death, it is in a secondary capacity. There is no treatment of this organ by injection of any extract, x-ray treatment, or extirpation which will have any effect on preventing sudden death. One member of the panel insisted that so-called status thymolymphaticus may be improved by x-ray treatment.

Whatever knowledge has been gained experimentally concerning the functions of the thymus are not applicable in clinical medicine to-day.—M. B. G.

THYROID

WILLIAMS, R. G. Further observations on the microscopic appearance and behavior of living thyroid follicles in the rabbit. *J. Morphol.* 65: 17. 1939.

Structures and changes constantly or intermittently visible in living grafts of thyroid gland studied with the transparent chamber technic were: nuclei, cement substance and terminal bars, proximal and distal cell borders, droplets in the cytoplasm and colloid, amount and character of colloid, fat droplets and granules in the cytoplasm, size and shape of follicles, degree of vascularization, and cycle of change. Most follicles were stationary at any given time, or underwent slow oscillation between stages 1 and 2. These were considered an inactive reserve. All activity was greatly diminished by sodium iodide after the initial response, which in some cases resembled apocrine secretion. Thyreotropic factor accelerated activity; the first effects were similar to those produced by iodide. Subsequent effects were of two types, 1) in which there was loss of colloid and increase in height of cells; 2) in which there was great increase in colloid and little increase in cell height. In follicles in separate grafts in the same animal the response was predominantly of a single type. The construction of an improved transparent chamber for transplantation is described.—*Abstract Wistar Institute Bibliographic Service.*